# DISSERTATION

# ON

# ESTIMATION OF HIGH SENSITIVITY C-REACTIVE PROTEIN AND LIPOPROTEIN[a] IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

**Dissertation submitted to**

**THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY**

**In partial fulfillment of the regulations**

**for the award of the degree of**

**M.D. BIOCHEMISREY- BRANCH – XIII**


**CHENNAI MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,**
**IRUNGALUR, TRICHY- 621 105**

**THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY**

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**APRIL - 2016**

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This is to certify that this dissertation entitled **"ESTIMATION OF HIGH SENSITIVITY C-REACTIVE PROTEIN AND LIPOPROTEIN[a] IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE"** is a bonafide original work of **Dr.SYNTHIYA.A** in partial fulfillment of the requirements for M.D Branch –XIII (Biochemistry) Examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in APRIL - 2016. The period of study was from 2013 – 2016.

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# DECLARATION

I, **Dr.SYNTHIYA.A**, solemnly declare that the dissertation titled **"ESTIMATION OF HIGH SENSITIVITY C-REACTIVE PROTEIN AND LIPOPROTEIN[a] IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE"** was a bonafide work done by me at Chennai Medical College Hospital and Research Centre ,Irungalur, Trichy during January 2014 - December 2014 under the guidance of my Professor and Head of the Department Dr.Kalavathy Ponniraivan M.D.,

This dissertation is submitted to Tamil Nadu Dr.M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D Degree (Branch-XIII) in Biochemistry.

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INTRODUCTION

Chronic obstructive pulmonary disease(COPD) has been defined by the global initiative for chronic obstructive lung disease(GOLD) Global initiative of chronic Obstructive Lung Disease[1] as a disease state characterized by air flow limitation that is not fully reversible, usually progressive and is associated with an abnormal inflammatory response of the lung to inhaled noxious particles or gases. Chronic obstructive pulmonary disease includes[2-4]

1.EMPHYSEMA: A bronchial pulmonary enlargement of air spaces distal to the terminal bronchiole along with destruction of their walls without any fibrosis

2.CHRONIC BRONCHITIS: Chronic bronchial sputum in her secretion resulting in chronic expectoration, coughing out of sputum on most days during atleast 3 consecutive months in 2 successive years).

3.SMALL AIRWAY DISEASE: Narrowing of small bronchioles.

GOLD estimates that Chronic obstructive pulmonary disease is the 4th most common cause of death worldwide[5] and it is predicted that it will be the 3rd most common cause of death in future (2020).

In India, after pulmonary tuberculosis, Chronic obstructive pulmonary disease remains the second most common disorder affecting the lung.

Chronic obstructive pulmonary disease is frequently seen in middle aged individuals. It is more commonly seen in males due to increased prevalence of smoking in our males.

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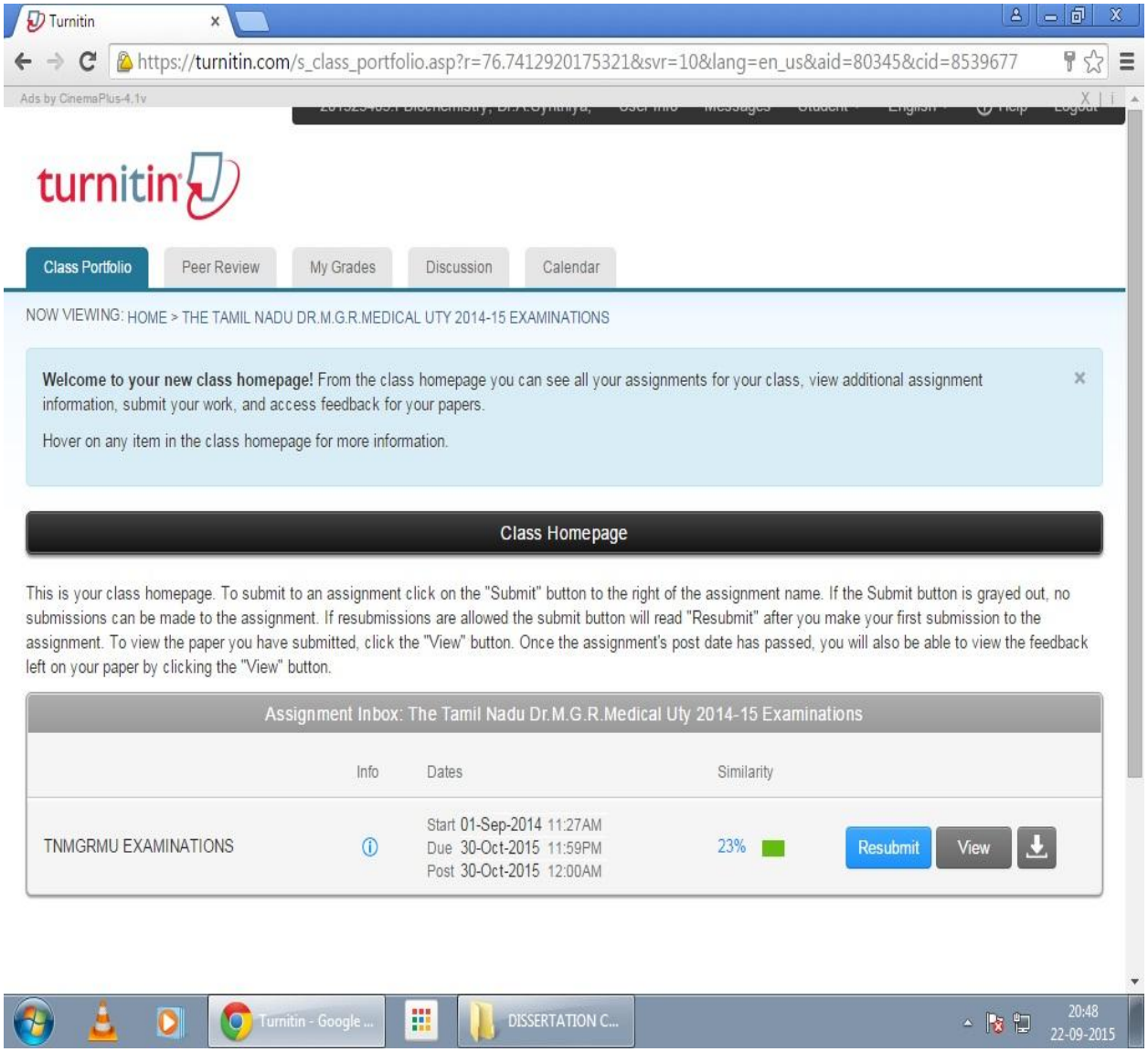
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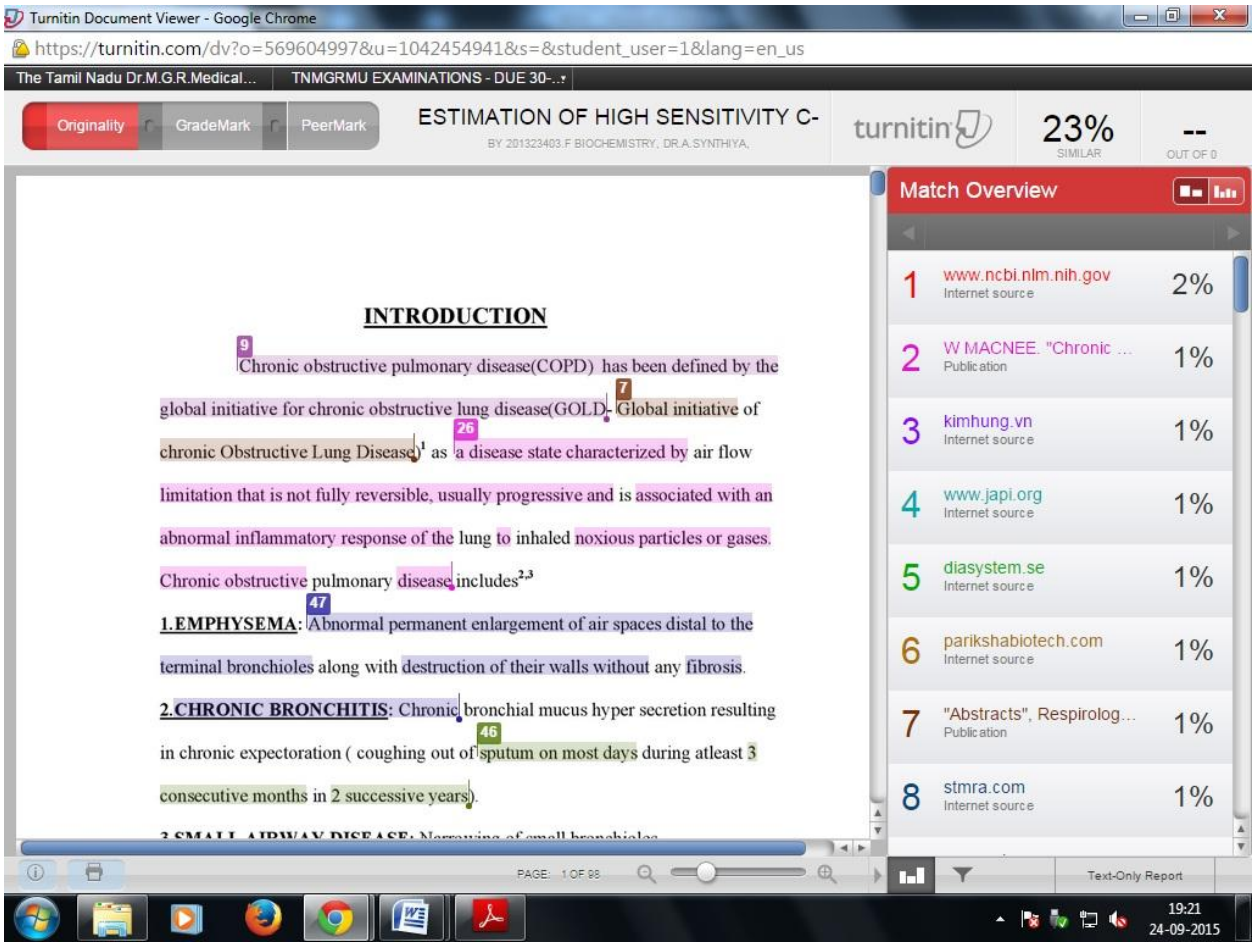
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# CONTENTS

# ABBREVIATIONS

1. **COPD** Chronic Obstructive Pulmonary Disease
2. **GOLD** Global Initiative For Obstructive Lung Disease
3. **Hs-CRP** High sensitivity C-reactive protein
4. **FEV1** Forced Expiratory Volume In 1 Second
5. **FVC** Forced Vital Capacity
6. **TLC** Total Lung Capacity
7. **RV** Residual Volume
8. **PAH** Pulmonary Arterial Hypertension
9. **ATS** American Thoracic Society
10. **APR** Acute Phase Response
11. **MRFIT** Multiple Risk Factor Intervention Trial
12. **WHI** Women Health Initiative Study
13. **Lp(a)** Lipoprotein(a)
14. **T. Cholesterol** Total cholesterol
15. **TGL** Triglycerides
16. **LDL-C** Low density lipoprotein Cholesterol
17. **HDL- C** High density lipoprotein Cholesterol
18. **VLDL-C** Very low density lipoprotein Cholesterol

## ABSTRACT

## ESTIMATION OF HIGH SENSITIVITY C-REACTIVE PROTEIN AND LIPOPROTEIN[a] IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

**BACKGROUND:**

Chronic obstructive pulmonary disease remains the second most common disorder affecting the lungs, next only to pulmonary tuberculosis in India. Recently Hs-CRP measuring methods have made it possible to assess this protein even in lower levels of inflammation. Patients affected by COPD have an risk of atherothrombotic acute events. Hypercoagulable state is assessed by measuring the Lp(a).

**AIMS OF THE STUDY:**

To study the levels of Hs-CRP and Lp(a) in COPD patients and to assess their correlation with the severity of the disease and the risk of atherosclerotic disease respectively.

**MATERIALS AND METHODS**:

Patients with clinically diagnosed and spirometrically confirmed cases of COPD were included in the study and classified based on GOLD staging. This was a case-control study with sample size of 50 cases and 30 controls, conducted in Chennai medical college hospital and research centre during January 2014 – December 2014. Serum Hs-CRP levels and Lp(a) levels were measured by Immunoturbidimetry method.

**RESULTS:**

The levels of Hs-CRP were significantly elevated in COPD patients. There exists a positive correlation with severity of the disease, obese individuals and smokers. There was no association of Hs–CRP levels with age and duration of the disease. Circulating Lp(a) levels are higher in COPD patients may thus be regarded as a valid biomarker of atherothrombotic acute events. There is no association between mean Lp(a) levels and the spirometry staging, BMI, smoking, disease duration.

**CONCLUSION:**

Serum Hs-CRP and Serum Lp(a) levels may be used as a simple auxiliary marker in staging determining the severity and prognosis of COPD and in determining the risk of atherosclerotic disease in COPD patients respectively for early intervention.



**Key words:** High sensitivity C-reactive protein, Lipoprotein(a), Chronic Obstructive Pulmonary Disease, Immunoturbidimetry, Spirometry, GOLD staging.

# INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has been defined by the global initiative for chronic obstructive lung disease (GOLD- Global initiative of Chronic Obstructive Lung Disease)[1] as a disease state characterized by air flow limitation that is not fully reversible, usually progressive and is associated with an abnormal inflammatory response of the lung to inhaled noxious particles or gases. chronic obstructive pulmonary disease includes[2,3]

**1.EMPHYSEMA:** Abnormal permanent enlargement of air spaces distal to the terminal bronchioles along with destruction of their walls without any fibrosis.

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**3.SMALL AIRWAY DISEASE:** Narrowing of small bronchioles.

GOLD estimates that chronic obstructive pulmonary disease is the 6$^{th}$ most common cause of death worldwide[4] and it is predicted that it will be the 3rd most common cause of death in future (2020).

In India, after pulmonary tuberculosis , chronic obstructive pulmonary disease remains the second most common disorder affecting the lung.

Chronic obstructive pulmonary disease is frequently seen in middle aged individuals. It is more commonly seen in males due to increased prevalence of smoking in our setup.

It is equally prevalent both in rural and urban areas. Increasing urbanization and emergence of industries leading to air pollution and increased smoking among young people may have definite impact on the incidence of chronic obstructive pulmonary disease.

Some of the predisposing factors in childhood for development of chronic obstructive pulmonary disease in future includes low birth weight, malnutrition and recurrent respiratory tract infection.

In patients with Chronic obstructive pulmonary disease the most robust test in assessing the air flow limitation is the spirometry.[5]

A low FEV1 (FEV1< 80%) with FEV1/FVC ratio less than 0.7 and less than 15% reversibility of airflow obstruction post bronchodilator therapy is the diagnostic criteria for chronic obstructive pulmonary disease.

With the wide spread use of spirometry, identification of air flow obstruction is considered as a key factor in determining the disability of chronic obstructive pulmonary disease patients.

Patients with chronic obstructive pulmonary disease will experience a systemic inflammation. It is assessed by measuring the inflammatory mediators like C reactive protein.

Recently high sensitivity C-reactive protein (Hs-CRP) measuring methods have made it possible to assess this protein even in lower levels of inflammation.

Chronic obstructive pulmonary disease patients have an increased risk of atherothrombotic acute events, independent of smoking and other cardiovascular risk factors.

Bronchial inflammation spreads to the systemic circulation and is known to play a key role in plaque formation and rupture.

Systemic inflammation is the first common cause of the hypercoagulable state observed in copd[6].

Hypercoagulable state is assessed by measuring the lipoprotein(a).

## AIMS OF THE STUDY

1. To estimate the level of High Sensitivity C-reactive Protein in patients with Chronic Obstructive Pulmonary Disease.
2. To estimate the level of Lipoprotein[a] in patients with Chronic Obstructive Pulmonary Disease.

## OBJECTIVES OF THE STUDY

1. To assess the correlation of High Sensitivity C-reactive Protein levels with various stages of COPD.
2. To evaluate the correlation of Hs-CRP in COPD patients with respect to other variables such as age, sex, smoking habits and disease duration.
3. To assess the correlation of smoking habits with severity of disease.
4. To assess the correlation of BMI levels with severity of disease.
5. To assess the correlation of Lipoprotein(a) levels with Lipid profile.
6. To prove the use of Hs-CRP in determining the severity of COPD and better control of disease prognosis.
7. To prove the use of Lipoprotein(a) in determining the risk of atherosclerotic disease in COPD patients.

# REVIEW OF LITERATURE

From the time of **Laennec.**, et al throughout the first half of the 20th century, mechanical explanations of chronic obstructive pulmonary disease dominate.

As early as in 1905 **OPIE et al.,** suggested that serine proteases and antiproteases imbalance plays an important role in pathophysiology of emphysema.

In 1956 Medical council of research first used the terminology chronic bronchitis in patients with chronic cough with sputum production.

In 1959 the causal relation between the smoking and persistent cough with expectoration was explained by **Higgins.,et al.**

In 1960 **Owen and Campbell et al.,** observed the pathological changes in airway due to cigarette smoking.

In 1973 **Boughly et al,.** submitted a list of articles related to prognostic factors in COPD and importance of pulmonary function test in these patients.

In 2007 **Sanjamarevic et al.,**[61] concluded that levels of high sensitivity C-reactive protein were significantly higher in patients with COPD and hence proved that it will be a more sensitive marker than TNF alpha, CXCL-8 and big ET 1 in systemic circulation.

**Daianastolz et al.,**[67] in 2007 analysed the levels of copeptin, C-reactive protein, procalcitonin in acute exacerbation of COPD patients and all are found to be significantly elevated.

**Yannick MTA et al.,**[63] from Erasmus university in 2008 observed the predictive role of high sensitivity C-reactive protein for COPD in smokers.

In 2008-2009 **SAAlavi et al.,**[62] from Guilan university, Iran found a correlation between the levels of high sensitivity C-reactive protein based on the GOLD staging of COPD.

**Bridevaux et al.,**[66] in 2009 in his SAPALDIA cohort study (swiss study on air pollution and lung diseases in adults) correlated the elevated levels of high sensitivity C-reactive protein in association with fast decline of FEV1 and obese patients.

In 2010 **Lisa Tileman et al.,**[60] from Germany identified the distinct aspects of systemic inflammation in bronchial asthma and COPD by measuring the levels of high sensitivity C-reactive protein (significantly elevated in COPD) and IgE, blood eosinophils, fractional exhaled nitric oxide levels (elevated in bronchial asthma). He also suggested that these markers have replaced the role of spirometry when not available.

**Jack et al.,**[68] in 2010 concluded that levels of high sensitivity C-reactive protein and surfactant protein D levels are higher in acute exacerbation of COPD which indicates the infectious state in our body. So it can also be used as a guide in the treatment of acute exacerbation of COPD.

Recent study conducted in Maulana Azad medical college in New Delhi correlated the levels of Hs-CRP as a marker of functional disability in COPD patients.

**Filippo Luca Fimognari et al**[6]**.,** in 2008 identified the mechanisms of atherothrombosis in chronic obstructive pulmonary disease. Systemic inflammation plays a leading role in atherosclerotic plaque formation and rupture, but other mechanisms, such as platelet activation, coagulation and oxidative stress, can promote atherosclerosis in COPD.

**Stephan Van Eeden et al**.[56]**.,** in 2012 identified the relationship between lung inflammation and cardiovascular disease. Acute and chronic lung inflammation is an under recognized risk factor for cardiovascular disease. There are compelling epidemiological data to indicate that airway exposures to cigarette smoke, air

pollution particles, viral and bacterial pathogens are strongly related to acute ischemic events. This article, reviewed the epidemiological data for the relationship between lung inflammation and cardiovascular disease and provide plausible mechanistic pathways by which acute and chronic inflammation contributes to the development of acute cardiovascular syndromes.

**Archana Burman A[1], et al[57]** identified Lipoprotein(a) as a marker of coronary artery disease and its association with dietary fat. This study confirms that though TC, TG and LDL-C are important risk factors for CAD, Lipoprotein(a) shows a stronger association with CAD than these lipids. Hence Lipoprotein(a) may prove to be a better discriminator of CAD risk at an early age since its levels are alleged to be genetically determined.

**Fauzia Ashfaq, et al [58]** identified Lipoprotein(a) levels in relation to the severity of coronary artery disease in North Indian patients. This study confirms the necessity of Lipoprotein(a) and other risk factors to predict the severity of coronary atherosclerosis, suggesting that Lipoprotein(a) levels should be determined in patients with CAD, especially in normolipidemic individuals.

# CHRONIC OBSTRUCTIVE PULMONARY DISEASE

It is a preventable and treatable condition characterized by progressive air flow limitation that is not fully reversible. It includes a group of conditions that occurs due to pathological changes in large and small airways as well as lung parenchyma. It is due to abnormal inflammatory response in lungs to inhaled noxious particles and gases.

In clinical practice , the diagnosis of COPD should be thought in patients above 35yrs of age, those with chronic progressive symptoms (cough, wheeze, breathlessness) and other risk factors such as cigarette smoking and occupational or environmental dusts or gases.

## Prevalence in India:

In males the prevalence rate is 2.12 to 9.4% in North India compared to 1.4 - 4.08% in South India.[7,8.] In females the prevalence is less which is about 1.3-4% from North India and 22.5-2.7 % in South India.[9] In general the male female ratio is about 1.6:1.0

## COPD and its comorbidities:

1. Diabetes
2. Ischemic Heart Disease
3. Depression
4. Osteoporosis

5. Weight Loss

6. Peptic Ulcer Disease

7. Glaucoma

**Natural history and prognosis:**[10,11,12]

FEV1 declines at a rate of 20-30ml/hour in non-smokers, whereas in smokers the decline rate will be more than 50ml/hour. FEV1 is a strong predictor of survival. Only less than 50% of patients whose FEV1 less than 30% may survive 5 years and above. Recent studies showed that the BODE index which includes FEV1, dyspnea, body weight, 6 minutes walk test are important in predicting the prognosis than FEV1 alone.

## RISK FACTORS:

### Smoking:

Most commonly identified correlate with chronic bronchitis is cigarette smoking. The mortality rate varies with the dose response curve with pack years of smoking.[13] When compared to nonsmokers, there is a 25% increase in mortality in smokers. The mortality in pipe and cigar smokers is lower than the cigarette smokers. Filter tip cigarettes are comparably less harmful.

Incidence of the disease decreases if smoking is stopped early in the course of the disease.[14,15] Cessation of smoking do not normalize the lung functions, instead it slows down the deterioration.

In males the loss of FEV1 in excess of normal decline with aging is 9ml per year for each pack year of smoking. In females, the rate of decline in FEV1 is 6ml.[16]

Prolonged smoking impairs the ciliary motility, macrophages accumulate in higher quantities in respiratory bronchioles which in turn releases proteinases and is responsible for destruction of extracellular matrix of lung. These macrophages recruit chemotactic factors which in turn attracts other inflammatory cells to lung and causes mucus gland hyperplasia. Cigarette smoke inhibits anti proteases and releases proteolytic enzymes, thereby destroying the alveolar wall.[17]

Passive smoking otherwise called as environmental tobacco smoke exposure[18,19] plays an important role in nonsmokers particularly among the women.

**Air pollution:**

Pollutants for air pollution includes exhausts from automobiles, industries and factories.[20,21,22] Smoke from solid fuel combustion such as dried dung, wood crop residues used in cooking in villages and slum areas also contributes to major source of air pollution in developing countries like India[23,24].

**Occupation :**

According to American thoracic society the occupational contribution to the burden of COPD is around 15%.[25,26] Various studies from all over the world quoted that occupation involving exposure to dust, gases, fumes as a major contributing factor to COPD. Chronic bronchitis is more prevalent among workers

exposed to organic or inorganic dusts. Occupational hazards include exposure to cadmium, toluene gas in plastic plants, construction and utility work.

**Recurrent respiratory tract infections**:

Lower respiratory infections in childhood is also postulated as a risk factor for the development of COPD.[27,28,29] This is due to the permanent damage or impaired lung growth. The risk of reduced lung function in future is high.

**Airway hyper responsiveness:**

Many patients with COPD also experiences airway hyperresponsiveness[30,31,32] which is a common association in bronchial asthma. The rate of reversibility in the degree of obstruction with bronchodilators is less than 15 %.

**GENETIC BACKGROUND:**

Aggregation of cases in families suggest that there is an established role for genetic factors in the pathogenesis of COPD. Occurrence of early onset COPD and decrease in the maximal expiratory flow rate among the nonsmoking first degree relatives of COPD supports the above said statement.

Polymorphism of genes involving protease – antiprotease balance, antioxidant function, inflammation and immune responses has been proposed.

Alpha 1 antitrypsin is a major antiprotease enzyme in the serum.[33] The synthesis of alpha one antitrypsin is regulated by a gene on chromosome 14q32. The most common deficient allele is PiZZ phenotype which is due to amino acid substitution of 342 Glutamic acid to lysine.[34]

This change results in spontaneous polymerization of polypeptide and impaired release of alpha one antitrypsin from the liver. This deficiency is rare in Asian and African descendants, whereas more common in European population (1 in 2000 to 1 in 7000). About 2 % of the patients with emphysema is due to alpha-1 antitrypsin deficiency.[35]

Patients usually presents with premature bronchiectasis or chronic bronchitis. More than 80 percent of the patients have autosomal recessive inheritance. Decline in FEV1 is around 100-130ml per year for smokers ,50-80ml per year for exsmokers and nonsmokers.

Panacinar emphysema is the most common type seen with predominant involvement of lower lobes of the lung. Smoking is an important cofactor in the development of COPD in patients with alpha one antitrypsin deficiency. They are also at an increased risk for cirrhosis of liver.

## PATHOLOGY:

Pathological changes are seen in larger and smaller airways as well as in terminal bronchioles. Small airways are considered as important site of airflow limitation.[36,37] Various reasons which are responsible for narrowing of lumen of small airways are goblet cell hyperplasia, edema, mucosal and submucosal inflammatory cells, peribronchial fibrosis, smooth muscle hypertrophy and mucus plugs.[38]

In larger airways hypertrophy of submucosal mucous glands is seen. Reid index is a measure of thickness of submucous glands to that of bronchial wall . In normal individuals the range is between 0.44 to 0.60, whereas in chronic bronchitis it is between 0.34 -0.53.

Emphysema starts as an increase in both number as well as size of the alveolar fenestrations and results in destruction of the septa. It also destroys the attachments of the septa to the terminal bronchioles. The site of destruction varies in different types of emphysema. In centriacinar it predominantly involves respiratory bronchioles, whereas panacinar involves both central as well as peripheral bronchioles.

## PATHOGENESIS AND PATHOPHYSIOLOGY:

Central to the pathogenesis is found to be an enhanced inflammatory process in response to inhaled particles and gases.[39,40] The pathogenesis includes various processes

1. Increased airway inflammation[41]
2. Increased protease burden-decreased anti protease function[42]
3. Oxidant –anti oxidant imbalance (oxidative stress)
4. Defective lung repair mechanisms

Chronic exposure to smoke, fumes, dusts results in inflammatory recruitment of inflammatory cells within the terminal air space of lungs. These cells results in destruction of walls and extra cellular matrix of the lungs.

Persistent reduction in the forced expiratory flow is the defining feature in case of COPD.

Other typical features are

1. Increased airway resistance
2. Increased residual volume
3. Increased RV/TLC
4. Decreased inspiratory capacity
5. Maldistribution of ventilation.

## Airflow obstruction:

Balance between the elastic recoil of the lungs that promote the flow and the resistance of airway that limits the flow contribute to the airflow during forced exhalation.

As the cross sectional area of the airway falls due to destruction the resistance increases, and the expiratory flow also decreases as the volume occupied decreases due to loss of elastic recoil and loss of radial traction of airways. In early stages the abnormality in airflow limitation is seen only at lung volumes at or below the FRV.

It is possible to distinguish between emphysema and small airway pathology only theoretically, since emphysema is due to decreased elastic recoil and small airway disease is due to increased airway resistance as a cause of reduced FEV1. Clinically it is more difficult to differentiate between these two as it co exist in most of the patients.

FEV1 correlation is better with small airway pathology when compared to emphysema. FEV1 remains a good predictor because $PaO_2$ usually remains near normal till FEV1 decreases up to half of the predicted level. Very low levels of FEV1 can be still associated with normal $PaO_2$. Usually $PaCO_2$ is not elevated until the FEV1 is less than 25% of predicted value.

**Maldistribution of ventilation**:

COPD is a heterogenous disease, since it affects both airways as well as lung parenchyma. This heterogeneity can be revealed by xenon gas ventilation.

## MIGET classification of COPD:

Type A- high ventilation perfusion ratio-emphysema

Type B-low ventilation perfusion- chronic bronchitis

But most of the COPD patients will have neither type A or B, they will have both high and low perfusion areas. The reduction of $PaO_2$ is mainly due to Ventilation/Perfusion mismatch, shunt is minimal. So moderate concentration of $O_2$ can correct the hypoxemia in COPD. If it is not getting corrected then other causes such as pulmonary embolism, shunting should be considered.

## Hyperinflation:

It is defined as

1. increased FRV.
2. increased residual volume to total lung capacity.
3. decreased inspiratory capacity to total lung capacity.

Though hyperinflation may be some times beneficial, adverse effects are more.

The adverse effects are due to

1. Loss of apposition zone,between the diaphragm and abdominal wall, so pressure cannot be transmitted for effective respiration.

2. Flattened short diaphragm muscles are not able to generate inspiratory pressures.

3. Increased tension required to generate transpulmonary pressure.

In those with hyperinflation the inspiratory capacity will be reduced.

Inspiratory capacity can be used as a prognostic significant value independent of the FEV1.

Recently lung cells senescence has been involved in the pathogenesis of emphysema.

## Pathophysiology of exacerbation:

Exacerbation of COPD are associated with a further increase in inflammatory response in the lungs predominantly involving neutrophils. These response may be triggered by bacterial or viral infection or by pollutants. This worsens the existing ventilation perfusion mismatch leading to respiratory failure and death.

## Lesions of the vessels in COPD:

There is no specific change in the vessel wall of the patient with COPD, sometimes atheromata may be seen. Pulmonary hypertension develops during late phase of the disease. These changes are mostly secondary to vascular shunting and increased intimal fibrosis.

## Pulmonary circulation in COPD:

In later stages of the disease pulmonary arterial hypertension develops, along with the development of hypoxemia, hypercapnia. It is the important complication of COPD. It is associated with development of right ventricular hypertrophy and worse prognosis.

## Abnormal blood gas tension:

## Hypoxemia:

Since hypoxemia is a potent vasoconstrictor , $PaO_2$ has an inverse relationship with the development of pulmonary arterial hypertension. Increasing arterial desaturation worsens the pulmonary pressure. Pulmonary artery pressure rises suddenly during REM sleep because of the relative hypoxemia and recurrence of this nocturnal pulmonary hypertension is inturn responsible for the changes in pulmonary hypertension.

**Hypercapnia:**

There exists a direct relation with the $PaCO_2$ and pulmonary artery pressure. This is probably due to hyperventilation induced hypercapnia or hypoxia induced pulmonary hypertension.

**Acidemia:**

Combination of both hypoxia and hypercapnia results in pulmonary hypertension in patients with COPD. So for given $PO_2$ the mean Ppa is higher with increasing hydrogen concentration.

**Effects of abnormal pulmonary mechanics:**

Changes in the pulmonary resistance results from increase in airway resistance matching with decrease in FEV1.

**Effects of increased cardiac output:**

Even minor increments in the cardiac output that occurs during exercise significantly rises the pulmonary arterial pressure.

**Effects of blood viscosity:**

Chronic hypoxemia which in turns develops polycythemia due to increased production of erythropoietin contributes to increasing blood viscosity thereby increasing pulmonary arterial hypertension.

## Corpulmonale:

Corpulmonale is defined as right ventricular hypertrophy and dilatation secondary to diseases of the lung parenchyma/vasculature or both. The prevalence of corpulmonale is higher in patients with hypercapnia, hypoxemia, polycythemia and in those with reduced FEV1. In clinically stable patients inspite of elevated pulmonary arterial pressure the right ventricular contractility is maintained.

In contrast, in patients with respiratory failure the right ventricular contractility is decreased . Edema in the late stages of the disease may not be entirely due to right ventricular failure, other causes should be ruled out. Due to hypoxemia and hypercapnia there will be a reduction in the renal function due to decreased blood flow which leads to changes in salt water balance. Decreased blood flow may be due to inappropriate arginine vasopressin levels or neurally mediated catecholamine release. Thus the development of peripheral edema in COPD involves a complex hemodynamics with multiple interactions.

Other grave risk factors include marked hypoxemia, increased pulmonary artery pressure and decrease in carbon monoxide transfer. In patients with right ventricular failure , the prognosis will be poor and mortality rate will be high up to 65-80%.

## INVESTIGATIONS:

## Radiology:

## Chest x-ray:

In chronic bronchitis, parallel line opacities are seen which are indicative of bronchial wall thickening.[43,44]

The radiographic features of emphysema includes:

- Overinflation of the lungs
- Low flattened diaphragm- the border of diaphragm lies below the 7th rib.
- Height of the lung is greater than 30 cm
- An obtuse costophrenic angle may be seen.
- Vertical and narrowed heart shadow is seen (tubular heart).
- Reduction in size and number of pulmonary vessels in the periphery of the lung.
- Hilar vessels are enlarged.
- In lateral chest x-ray there is an increase in the retrosternal space (>2.54 cm).
- Presence of bullous lesions is an important evidence of emphysema.

Bullae may present as a stable lesion. Sometimes, it may enlarge massively sufficient enough to cause the collapse of the entire lung. It is called as vanishing lung syndrome. Pneumothorax is the important differential diagnosis. Bullae may rupture to produce secondary pneumothorax.

 In fluoroscopy , low and flat diaphragm is seen. A paradoxical upward movement during inspiration may be noted.

**Computed tomography:**

It has greater sensitivity and specificity in the diagnosis of emphysema when compared to chest x-ray.[45,46] It is helpful in the evaluation of bullous lesions of the lung. It is seen as areas of low attenuation without Obvious margins. Attenuation and pruning of the vessels can be detected.

A decrease in the CT lung density signifies the presence of microscopic emphysema.

Bronchograms may show irregular, narrowed and distorted bronchi.

**Spirometry :[47]**

It is the most important test in assessing the air flow limitation. It is helpful in arriving at a diagnosis of COPD and also in predicting the severity of the disease and in further follow up of patients.

The major abnormalities include reduction in FEV1 and in the ratio of FEV1/FVC .

The presence of a post bronchodilator FEV1 <80% along with FEV1/FVC ratio <0.7 indicates the presence of airflow obstruction that is not totally reversible.

Assessment of reversibility to bronchodilators is done in COPD patients to differentiate it from bronchial asthma . It is also important in identifying the post bronchodilator FEV1 which is a better predictor of the prognosis.

According to ATS and GOLD guidelines a change in FEV1 of more than 200 ml and a percentage change of more than 12% is considered as significant reversibility but according to British thoracic society guidelines change in FEV1 50% above the base line is considered significant.

Around 30% of COPD patients may show significant reversibility with bronchodilator therapy.

**Arterial blood gas analysis:**

Useful in assessing degree of hypoxemia and hypercapnia.

Usually seen once FEV1< 50%

Blood gas abnormalities may occur during exercise and sleep and during exacerbations.

Though pulse oximetry is commonly used , it is not to be considered as a replacement for ABG.

## CRP an inflammatory marker:

Inflammation is considered as a protective response of vascular connective tissue to external injury or stimuli . It is usually associated with the release of inflammatory mediators like prostanoids, vasoactive amines, cytokines and reactive oxygen species.

The term acute phase response (APR)[48] is described to encompass all the changes occurring in various organs in response to systemic inflammation. It is a non specific response initiated by various stimuli like burns, surgical or physical trauma, irradiation and infection. Cytokines mediating this acute phase response are stumour necrosis factor alpha, interleukin 1 and interleukin 6 . One of the most fascinating fact of the APR is rise in acute phase proteins synthesized in the liver.

## Acute phase proteins:

### Positive acute phase proteins:

1.Protease inhibitors such as anti chymotrypsin and alpha 1 anti trypsin

2.Coagulation proteins such as fibrinogen, plasminogen, prothrombin and factor VIII.

3.Various complement proteins such as C2,C3,C4,C5,C1esterase inhibitor and plasminogen.

4.Transport and storage proteins such as haemopexin, ceruloplasmin, ferritin and haptoglobin.

5.Other positive APR ‘s are CRP, procalcitonin, serum fibronectin, alpha1 acid glycoprotein (orosomucoid), mannose binding lectin and serum amyloid protein.

**Negative acute phase proteins:**

1. Albumin
2. Pre albumin
3. Transthyretin
4. Transcortin
5. Transferrin
6. Antithrombin

CRP is a biological substance which was known as acute phase reactant for long back, originally described 70 years ago . Now it is used as an inflammatory marker. It was named so because of its interaction with phosphoryl choline and lipoteichoic acid found on pneumococcus.

CRP is the best known of the acute phase protein since it is regularly used as a marker of systemic inflammation in clinical settings[49]. They may increase from 1 mcg/ml to 500mcg/ml in severe inflammation.[50]

It is released in excess amount within 6 hours of an acute inflammatory stimulus. Doubling time in plasma occurs atleast every 8 hours. It attains the peak concentration after 50 hours.

The plasma concentration can fall almost as rapidly as 5-7hours plasma half life after appropriate treatment or removal of the inflammatory stimulus.

CRP is a member of the pentraxin family of proteins. It also includes homologues of similar size proteins such as serum amyloid protein (SAP) or larger proteins such as long pentraxins (PTX3) .

C-reactive protein specifically binds to phosphocholine present on the cell membrane of microbes and it activates the classical complement pathway and inturn opsonizes the ligands for phagocytosis. It down regulates the polymorphs and also neutralizes the platelet activating factor.

Although in the clinical context elevation of CRP is suggestive of infection or inflammation, it may also occur with various other conditions like obesity, malignancy and renal dysfunction. Conversely, a lack of elevation of CRP is seen during flairs of systemic lupus erythematosus as well as in patients with hepatic failure.

## Conditions with elevated CRP:

1.Bacterial infections such as pyelonephritis, meningitis and endocarditis.

2.Inflammatory diseases such as Rheumatoid Arthritis , Psoriatic Arthritis, Reiters Disease, Crohns Disease , Ankylosing Spondylitis And Familial Mediterranean Fever .

3.Malignancies such as lymphoma and sarcoma .

4.Necrotic infection such as acute pancreatitis, myocardial infarction and tumor embolization .

5. Other nonspecific conditions such as burns and fractures .

Levels of C-reactive protein in some conditions remained normal inspite of active inflammation which includes,

1. SLE
2. Dermatomyositis
3. Systemic sclerosis
4. Graft versus host disease
5. Leukaemia
6. Ulcerative colitis

The reason for this selective failure of raise in CRP in the above said conditions is unknown.

## CRP and cardiovascular disease:

Atherosclerosis is the process underlying cardiovascular disease. It is partly responsible for the chronic low level inflammation of the vascular endothelium . Inflammation is obvious at the site of plaque rupture[51].

Several studies like MRFIT[52] and WHI study in postmenopausal women support the above fact.

Apart from its use as an inflammatory marker it is also used in

1. Assessing the response to treatment in conditions such as rheumatoid arthritis where there will be a dramatic fall in CRP level following treatment.

2.To differentiate bacterial and viral infections (usually elevated in bacterial infection)

3. To avoid confusion between a disease flare and super infection in conditions such as systemic lupus erythematosus.

CRP levels remains unaltered by antipyretic or any other thermoregulatory factors. So it can be used as an adjunct to use temperature chart in clinical practice.

Recent works done in relation with cognitive assessment reveals that levels of CRP are high in those with impaired cognition.[53] The exact etiology for this is unknown. Further work is warranted in this field.

## High sensitivity CRP:

Though both measure the same substance in blood, Ultra sensitive or high sensitivity CRP[54] refers to the measurement of small changes in CRP concentrations which the standard test used for measuring CRP tend to miss.

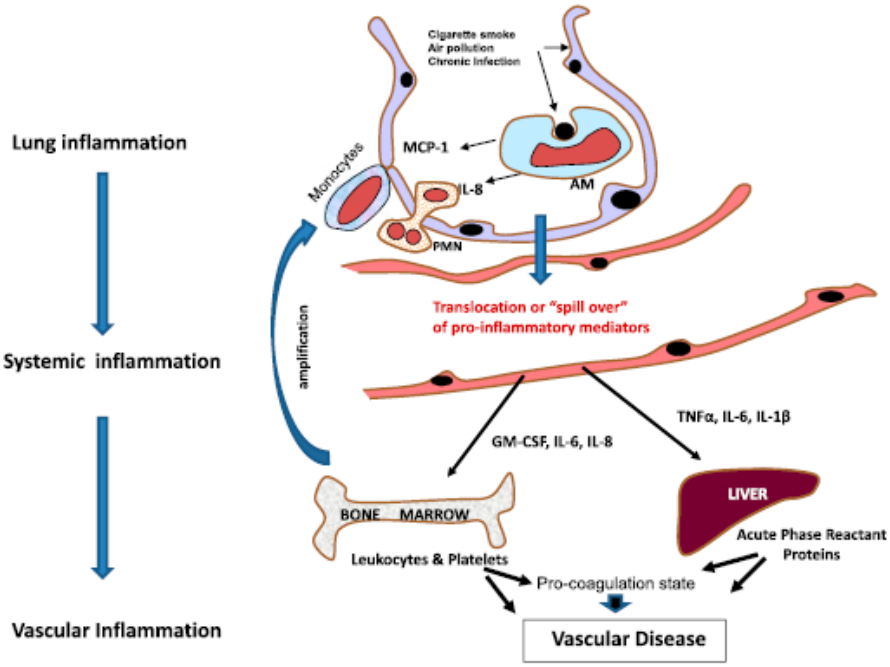
Standard test for CRP measures only values more than 10 mg/L . Values less than 10mg/L can be measured only by hs-CRP.[58] It is synthesized from the liver.

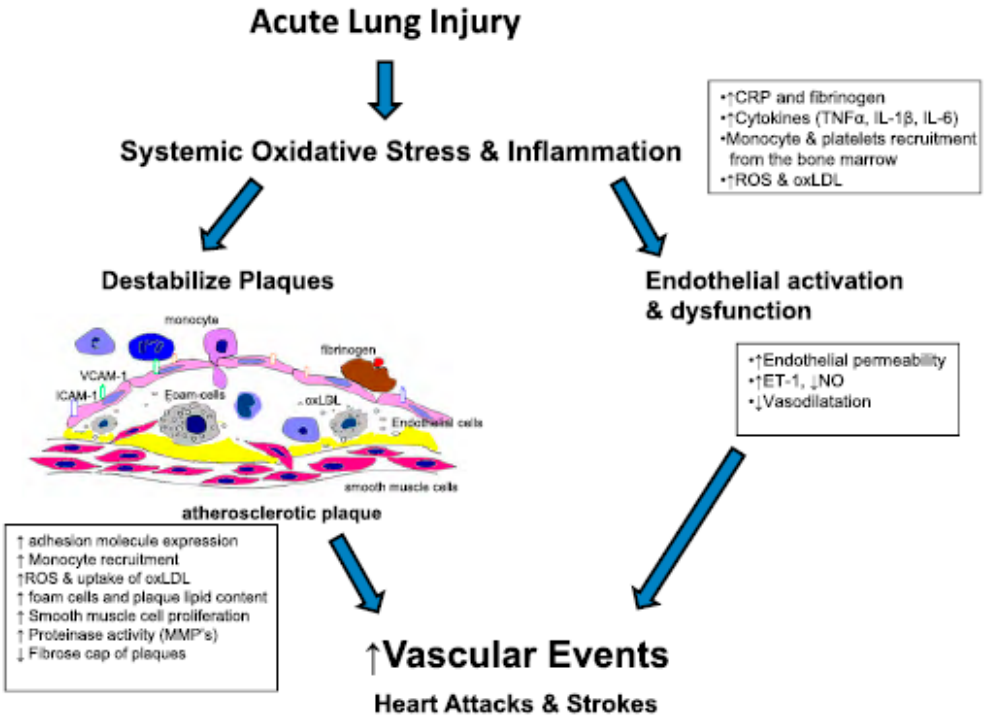
COPD is a chronic inflammatory disease of the lung, that is known to have systemic features, among which is an increased risk of cardiovascular disease. Cardiovascular diseases are the most potent killers particularly so in the advanced countries of the world. Cardiovascular disease is a leading cause of death in patients with COPD.[70]

## Pro-atherothrombotic mechanisms in COPD[6]

Four factors seem to be pathogenetically important:

1) Chronic systemic inflammation,
2) Hypercoagulable state,
3) Platelet activation and
4) Oxidative stress


Lung inflammation
Systemic inflammation
Vascular Inflammation
Cigarette smoke
Air pollution
Chronic infection
MCP-1
IL-8
AM
Monocytes
PMN
amplification
Translocation or "spill over"
of pro-inflammatory mediators
GM-CSF, IL-6, IL-8
TNFα, IL-6, IL-1β
BONE MARROW
Leukocytes & Platelets
LIVER
Acute Phase Reactant
Proteins
Pro-coagulation state
Vascular Disease


Acute Lung Injury
Systemic Oxidative Stress & Inflammation
•↑CRP and fibrinogen
•↑Cytokines (TNFα, IL-1β, IL-6)
•Monocyte & platelets recruitment from the bone marrow
•↑ROS & oxLDL
Destabilize Plaques
Endothelial activation & dysfunction
•↑Endothelial permeability
•↑ET-1, ↓NO
•↓Vasodilatation
monocyte
fibrinogen
VCAM-1
ICAM-1
Foam-cells
oxLDL
Endothelial cells
smooth muscle cells
atherosclerotic plaque
↑ adhesion molecule expression
↑ Monocyte recruitment
↑ROS & uptake of oxLDL
↑ foam cells and plaque lipid content
↑ Smooth muscle cell proliferation
↑ Proteinase activity (MMP's)
↓ Fibrose cap of plaques
↑Vascular Events
Heart Attacks & Strokes

## Systemic inflammation:

In the context of the complex and multifactorial pathogenesis of atherothrombosis, low grade systemic inflammation is one of the crucial mechanism in plaque formation and rupture. Alveolar macrophages, bronchial epithelial cells and lymphocytes, which are implicated in bronchial and alveolar inflammation, produce interleukin (IL)-6 and IL-1β.

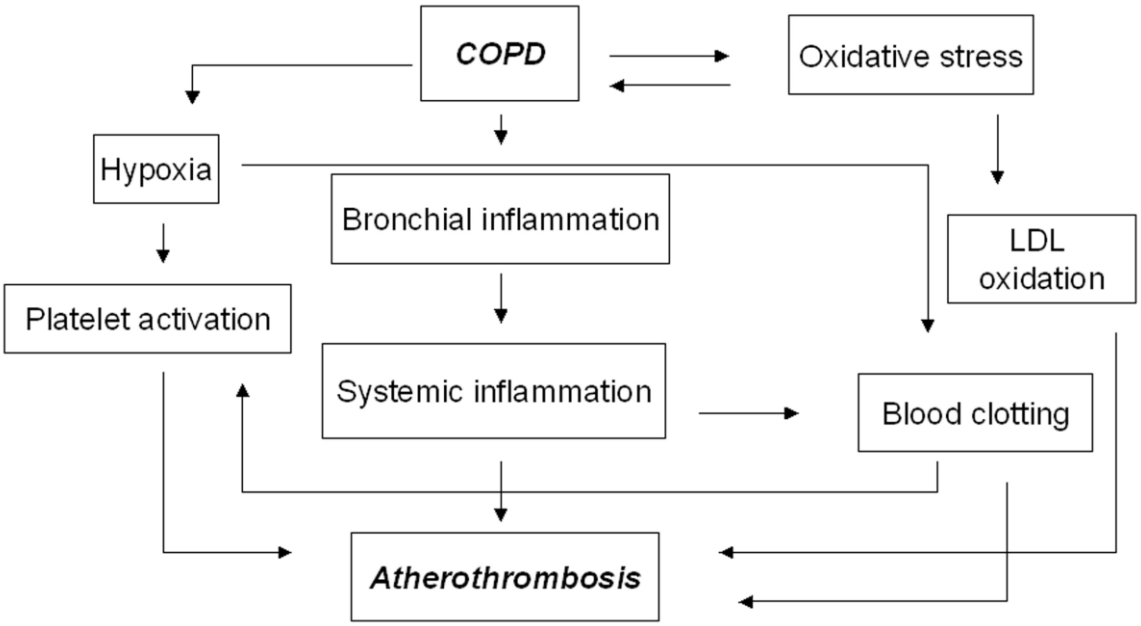
These cytokines, inducing local pro-inflammatory changes, "spill-over" into the systemic circulation and stimulate hepatocytes to synthesize inflammatory mediators.

## Hypercoagulable state:

The increased procoagulant activity in COPD may primarily result from inflammation. Inflammation can trigger coagulation by promoting tissue-factor gene expression in endothelial cells.

Hypoxia also could reduce endothelial thrombomodulin expression or activate factor X. Coagulation, in turn, increases inflammation and both are

strongly implicated in the pathogenesis of atherothrombosis.




## Platelet activation:

Platelet stimulation may result from clotting activation with thrombin generation, that, in turn, to enhance platelet thromboxane biosynthesis.

## Oxidative stress:

The development of COPD is associated with oxidative stress and reduced antioxidant properties. Hydrogen peroxide (H2O2) in exhaled breath condensate is a marker of oxidative stress in the lungs and have been found to be elevated in COPD patients irrespective of smoking status.

Oxidative stress can promote the peroxidation of polyunsaturated fatty acids. Pulmonary oxidative stress "spreads out" to the circulation and becomes a systemic alteration.

COPD is now recognized as a systemic inflammatory disease that may adversely affect the arterial district, predisposing patients to an increased risk of atherosclerotic plaque formation and rupture. Systemic inflammation plays a leading role in this process, but other mechanisms, such as platelet activation, coagulation and oxidative stress, can promote atherosclerosis in COPD.

Elevated serum lipoprotein(a) (Lp[a]) is an independent predictor of coronary artery disease (CAD) and myocardial infarction, intermittent claudication, cerebrovascular disease, and peripheral vascular disease.[72]

## Lipoprotein(a):-

Lp(a) is composed of an LDL particle which, through its apolipoprotein B100 (apoB100) moiety, is covalently bonded to a glycoprotein molecule known as apolipoprotein(a) [apo(a)]. High Lipoprotein[a] serum concentration have been associated with atherosclerotic disease and is considered as a strong independent risk factor for cardiovascular disease and cerebrovascular disease in COPD patients**.** In neumours trials patients with Lp[a] levels above 30mg/dl had markedly increased risk of atherosclerotic disease[57,58]**.**

## Method Of Measurement :

IMMUNOTURBIDIMETRY

.

# MATERIALS AND METHODS

The study was conducted at the Department of Biochemistry at Chennai medical college hospital and research centre during JANUARY 2014 – DECEMBER 2014.

## TOTAL NUMBER OF PATIENTS INCLUDED IN THE STUDY :

Case -50 patients (both males & females).

Control – 30 patients (both males & females).

## STUDY DESIGN:

This is a case control study.

## INCLUSION CRITERIA:

COPD patients whose diseases were diagnosed by specialists and confirmed by spirometry (as gold criteria).

## EXCLUSION CRITERIA:

1.Patients had a history of asthma, connective tissue disorders like [RA,SLE ], Inflammatory disease [inflammatory bowel disease] and malignancy.

2. Patients had acute or chronic renal failure, thyroid disorders, acute infection, stroke, and diabetic ketoacidosis.

## ETHICAL CONSIDERATIONS:

Necessary approval to conduct the study at the Chennai medical college hospital and research centre was obtained from institutional ethical committee. Patients were given an explanation about the purpose of the study and informed written consent was obtained, confidentiality about their results was assured. Their participation was optional.

## COLLECTION OF SPECIMENS:

5ml venous blood sample was collected under fasting and aseptic precautions in clot activator coated polypropylene tubes. Blood was centrifuged at 3500 rpm for 10 minutes and serum was separated. The specimens were freezed at -20°C for storage.

## ANALYSIS OF BLOOD SAMPLES:

The serum collected above was used for the estimation of the following parameters

## ESTIMATED PARAMETERS:

1) Serum High sensitivity C - Reactive protein by Immunoturbidimetry.

2) Serum Lipoprotein[a] by Immunoturbidimetry.

3) Serum Total Cholesterol by Enzymatic Cholesterol esterase method.

4) Serum Triglycerides by Colorimetric Enzymatic Test using GPO.

5) Serum High Density Lipoprotein Cholesterol by Direct enzymatic method.

## CALCULATED PARAMETERS:

1. Body Mass Index (BMI): $\frac{\text{Weight in Kg}}{(\text{Height in meters})^2}$

2. Very Low Density Lipoprotein cholesterol = TGL/5

3. Low Density Lipoprotein cholesterol = Total Cholesterol – ( LDL-c + VLDL-c )

# ESTIMATION OF SERUM HIGH SENSITIVITY C-REACTIVE PROTEIN

## Methodology:

Latex enhanced Immunoturbidimetry.

## Immunoturbidimetry:

It is defined as the detection of light energy scattered towards a detector that is not in the direct path of transmitted light. Commonly available immunoturbidimeters are those which measure the scattered light at right angles to the incident light. It is the commonly used technique for protein assays such as Lipoprotein(a), C-reactive protein, rheumatoid factor, anti streptolysin O and immunoglobulins.

## Principle:

This CRP test is based upon the reactions between C-reactive protein (CRP) and latex-covalently bound antibodies against human CRP. CRP values are determined turbidimetrically using fixed-time measurement with sample blank correction.

The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 to 50 mg/L. The measuring temperature is 37°C. The assay can be performed on all instruments allowing turbidimetric measurements at 546 nm.

## Reagents:

A. Buffer – 30ml of TRIS buffer(0.05), pH :7.2, containing polyethyleneglycol and 0.09% sodium azide as preservative.

B. Latex reagent – 5.1 ml of polystyrene particles (0.5%) coated with goat antibodies anti-human-CRP serum in a glycine buffer (0.1 M, pH :8.2), containing Nacl (0.15M) and bovine serum albumin (0.5%).

Preservative: Sodium azide 0.075%

## Preparation of reagents:

Working Reagent is prepared with 1 part of Latex Reagent and 7 parts of Buffer Reagent.

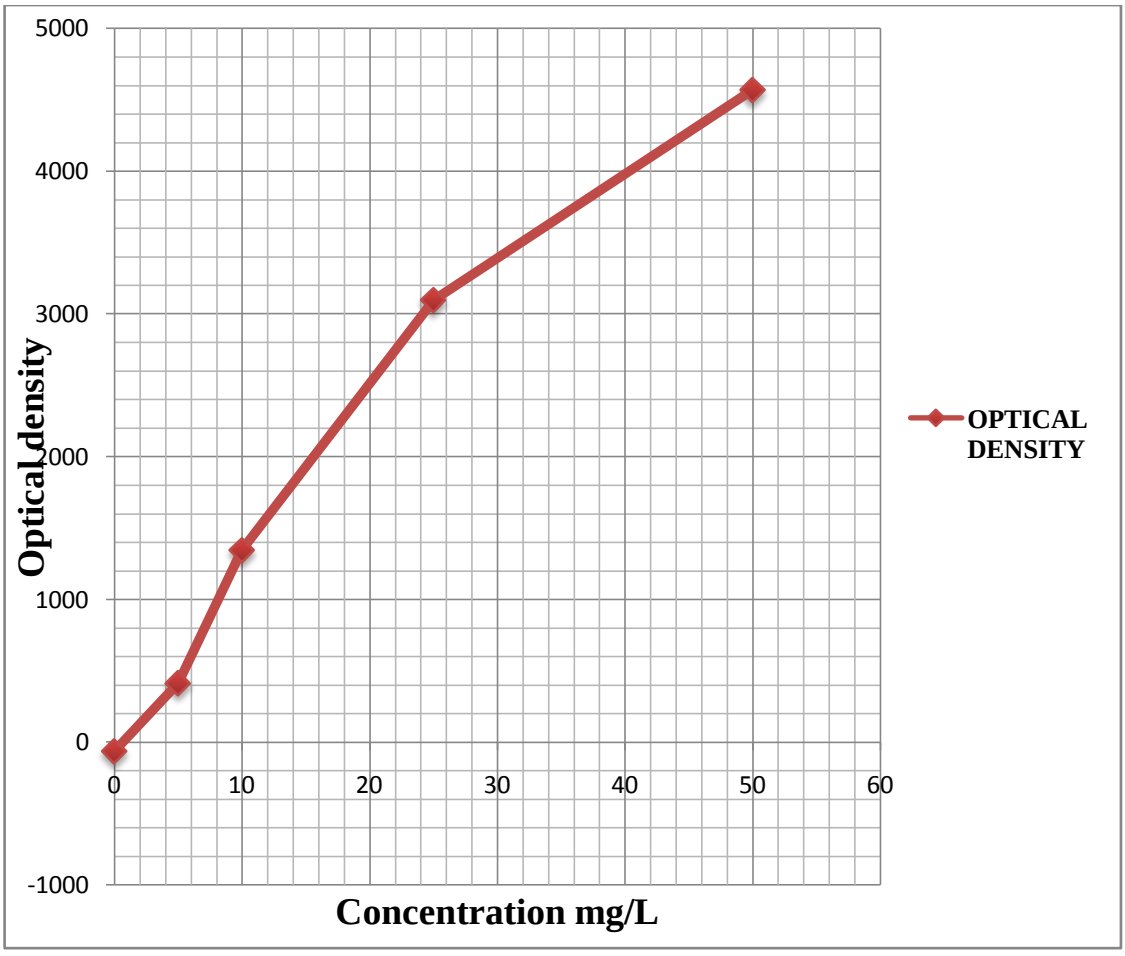
## Procedure:

The sample and the working reagent are brought to room temperature prior to use. Latex reagent should be gently shaken before each use.

| | |
|---|---|
| Calibrator 1 | 6.25 mg/L |
| Calibrator 2 | 12.5 mg/L |
| Calibrator 3 | 25mg/L |
| Calibrator 4 | 50 mg/L |

# CALIBRATOR CURVE OF HIGH SENSITIVITY

# C- REACTIVE PROTEIN



3 test tubes are taken and labeled them as blank (B), Calibrator (C), Test (T). 500µL of working reagent is added to 3 test tubes. 4 µL of distilled water is added to test tube labeled 'B' and 4 µL of serum is added to test tube labeled 'T' and 4 µL of Calibrator is added to test tube labeled 'C'. It is mixed and absorbance ($A_1$) is read immediately at 550nm and the mixture is incubated for 4 minutes ,the absorbance ($A_2$) is read.

| | **Blank** | **calibrator** | **Test** |
|---|---|---|---|
| Working Reagent | 500µL | 500µL | 500µL |
| Calibrator | -- | 4 µL | -- |
| Serum | -- | -- | 4µL |
| Distilled water | 4 µL | -- | -- |

## Calculations:

$$\frac{(A_2 - A_1)_{sample}}{(A_2 - A_1)_{calibrator}} - \frac{(A_2 - A_1)_{blank}}{(A_2 - A_1)_{blank}} \times \text{calib.conc} = \text{Sample concentration (mg/L)}$$

## Reference values:

Serum HsCRP < 6 - 8 mg/L.

# ESTIMATION OF SERUM LIPOPROTEIN(a)

## Methodology:

Latex enhanced Immunoturbidimetry.

## Principle:

The Lp(a) test is based upon the reactions between Lp(a) in the sample and latex-covalently bound antibodies against human Lp(a). Lp(a) values are determined photometrically.

## Reagents:

A. Buffer – 30ml of Glycine buffer , pH :8.0, containing protein stabilizers and 0.09% sodium azide as preservative.

B. Latex reagent – 4ml of a suspension of latex microparticles covalently bound antibodies against human Lp(a) in a glycine buffer (0.1 M, pH :8.2), containing Nacl (0.15M) and bovine serum albumin (0.5%). Preservative : Sodium azide 0.075%

C. Calibrator – lyophilised for 1mL. Human – based reference fluid. Preservative: sodium azide, 0.075% .

## Preparation of reagents:

Working Reagent is prepared with 1 part of Latex Reagent and 7 parts of Buffer Reagent.

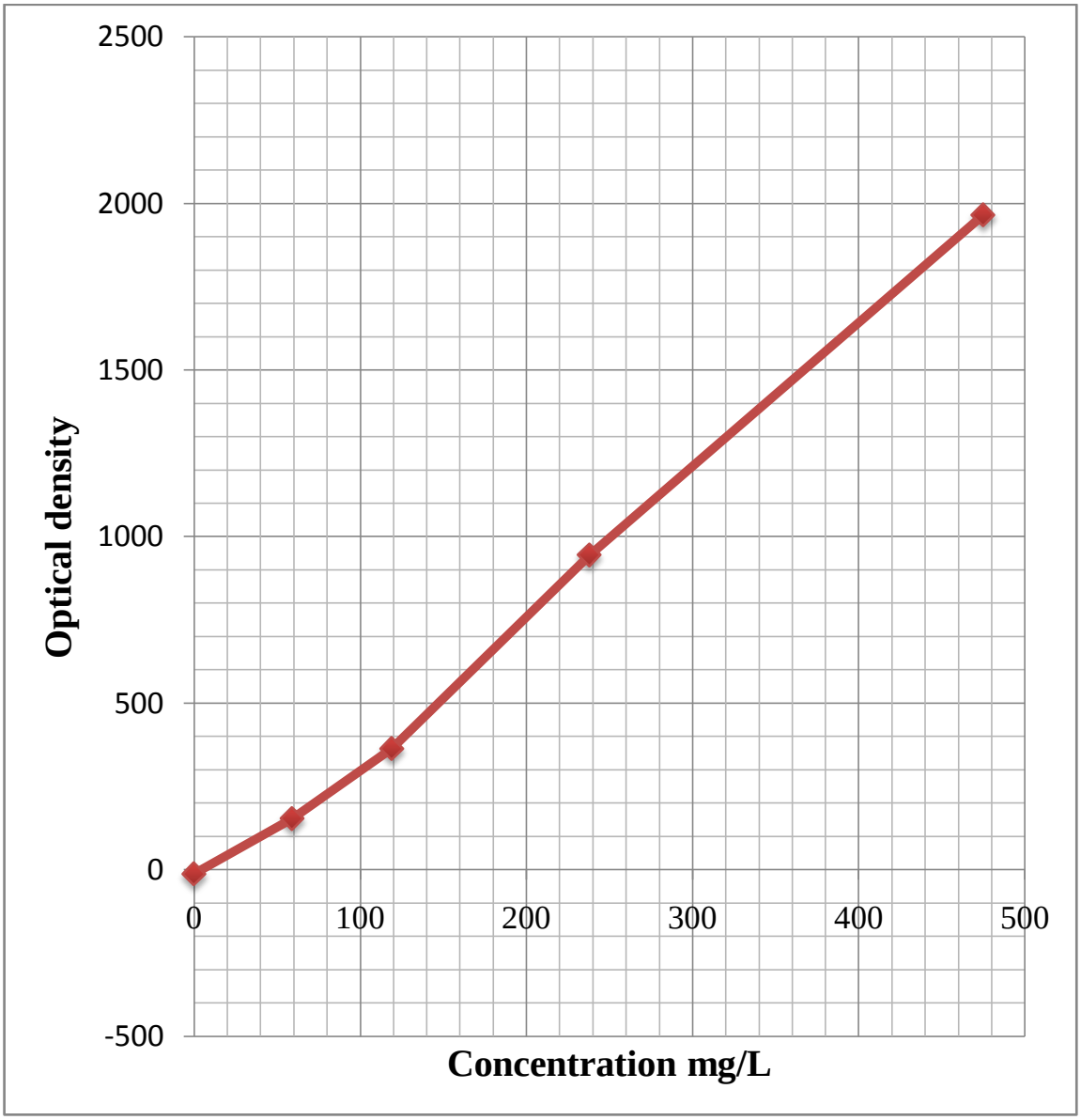
## Procedure:

The sample and the working reagent are brought to room temperature prior to use. Latex reagent should be gently shaken before each use.

| | |
|---|---|
| Calibrator 1 | 59 mg/L |
| Calibrator 2 | 119 mg/L |
| Calibrator 3 | 238 mg/L |
| Calibrator 4 | 475 mg/L |

3 test tubes are taken and labeled them as blank (B), Calibrator (C), Test (T). 500µL of working reagent is added to 3 test tubes. 4 µL of distilled water is added to test tube labeled 'B', 4 µL of sample is added to test tube labeled 'T' and 4 µL of Calibrator is added to test tube labeled 'C'. It is mixed and absorbance ($A_1$) is read immediately at 600nm and the mixture is incubated for 4 minutes ,the absorbance ($A_2$) is read.

# CALIBRATOR CURVE OF LIPOPROTEIN(a)



| | Blank | Calibrator | Test |
|---|---|---|---|
| Working Reagent | 500µL | 500µL | 500µL |
| Calibrator | -- | 4 µL | -- |
| Sample | -- | -- | 4µL |
| Distilled water | 4 µL | -- | -- |

## Calculations:

$$\frac{(A_2-A_1)_{sample}}{(A_2-A_1)_{calibrator}} - \frac{(A_2-A_1)_{blank}}{(A_2-A_1)_{blank}} \times \text{calib.conc} = \text{Sample concentration(mg/L)}$$

## Reference values:

Serum Lipoprotein(a) < 300 mg/L.

# LIPID PROFILE

## ESTIMATION OF SERUM TOTAL CHOLESTEROL

### Methodology:

Cholesterol Oxidase / Peroxidase method

### Principle of the method:

Cholesterol esters are hydrolyzed to produce cholesterol. Then, free cholesterol takes part in two coupled reactions that permit to measure cholesterol photometrically.

The reaction sequence is as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol.esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2} O_2 + H_2O \xrightarrow{\text{Chol.Oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

### Reagents:

| | |
|---|---|
| Pipes | 35mmol / L |
| Sodium cholate | o.5mmol / L |
| Phenol | 28mmol/L |

| | |
|---|---|
| Cholesterol esterase | >0.2 U/mL |
| Cholesterol oxidase | >0.1U/mL |
| Peroxidase | >0.8U/mL |
| 4-Aminoantipyrine | 0.5mmol/L |
| pH | 7.0 |

**Standard** :

Cholesterol 200mg/dl

**Storage**: 2ºC-8ºC

## Preparation of working solution:

Both the working reagent and the standard are supplied ready to use.

## Procedure:

The sample and the working reagent are brought to room temperature prior to use. Working reagent should be gently shaken before each use.

| | **Blank** | **Standard** | **Test** |
|---|---|---|---|
| Working Reagent | 1Ml | 1mL | 1mL |
| Distilled water | 10µL | -- | -- |
| Standard | -- | 10µL | -- |
| Sample | -- | -- | 10µL |

3 test tubes are taken and labeled them as blank (B), Standard (S), Test (T). 1 ml of working reagent is added to 3 test tubes. 10µL of distilled water is added to test tube labeled 'B', 10µL of sample is added to test tube labeled 'T' and 10µL of standard is added to test tube labeled 'S'. It is mixed and incubate the tubes for 10 minutes at room temperature. Read the absorbance at 500±10nm.

## Calculations:

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200 = \text{Sample concentration(mg/dl)}$$

## Reference values:

Serum Total Cholesterol:

Desirable : up to 200 mg/dl

Borderline High : 200 - 239 mg/dl

High : > 240 mg/dl.

# ESTIMATION OF SERUM TRIGLYCERIDES

## Methodology:

Glycerol-3-phosphate oxidase (GPO)

## Principle of the method:

Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4- chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

The reaction sequence is as follows:

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyaceton phosphate + } H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine + 4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoeimine + HCl +4 } H_2O$$

## Reagents:

| | |
|---|---|
| 4-chlorophenol | 4mmol / L |
| ATP | 2mmol / L |
| $Mg^{2+}$ | 15mmol/L |

Glycerolkinase(GK) ≥0.4 kU/L

Lipoprotein lipase(LPL) ≥ 2 kU/L

Peroxidase(POD) ≥ 2 kU/L

4-Aminoantipyrine 0.5mmol/L

Glycerol-3-phosphateoxidase(GPO) ≥0.5 kU /L

Good's buffer pH 7.2 50 mmol / L

**Standard :**

Triglycerides 200mg/dl

**Storage**: 2ºC-8ºC

## Preparation of working solution:

Both the working reagent and the standard are supplied ready to use.

## Procedure:

The sample and the working reagent are brought to room temperature prior to use. Working reagent should be gently shaken before each use.

| | **Blank** | **Standard** | **Test** |
|---|---|---|---|
| Working Reagent | 1ml | 1ml | 1ml |
| Distilled water | 10µL | -- | -- |
| Standard | -- | 10µL | -- |
| Sample | -- | -- | 10µL |

3 test tubes are taken and labeled them as blank (B), Standard (S), Test (T). 1 ml of working reagent is added to 3 test tubes. 10µL of distilled water is added to test tube labeled 'B', 10µL of sample is added to test tube labeled 'T' and 10µL of standard is added to test tube labeled 'S'. It is mixed and incubate the tubes for 10 minutes at room temperature. Read the absorbance at 546nm.

## Calculations:

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200 = \text{Sample concentration (mg/dl)}$$

To correct for free glycerol, subtract 10mg/dl from the triglycerides value calculated above.

## Reference values:

Serum Triglycerides:

| | |
|---|---|
| Desirable | : < 200 mg/dl |
| Borderline High | : 200 - 400 mg/dl |
| High | : > 400 mg/dl |

# ESTIMATION OF SERUM

# HIGH DENSITY LIPOPROTEIN CHOLESTEROL

## Methodology:

Direct enzymatic method.

## Principle:

Antibodies against human lipoproteins are used to form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement.

The reaction sequence is as follows:

$$\text{LDL, VLDL, Chylomicrons} \xrightarrow[\beta \text{ – lipoprotein antibodies}]{\text{Anti-human}} \text{Antigen – antibody complexes} + \text{HDL}$$

$$\text{HDL–Cholesterol} + H_2O + O_2 \xrightarrow{\text{CHE \&CHO}} \text{Cholest-4-en-3-one} + \text{Fatty acid} + H_2O_2$$

$$H_2O_2 + \text{F- DAOS} + \text{4- Aminoantipyrine} \xrightarrow{\text{POD}} \text{Blue Complex} + H_2O$$

## Reagents:

Reagent 1:

| | |
|---|---|
| Ascorbate oxidase | 2250 U/ L |
| Anti-human β - lipoprotein Antibody(sheep) | |
| Peroxidase | 2000 U/L |
| 4-Aminoantipyrine | 0.75mmol/L |
| Good's buffer pH 7.0 | 25 mmol / L |

Reagent 2:

| | |
|---|---|
| Good's buffer pH 7.0 | 30 mmol / L |
| Cholesterol esterase(CHE) | 4000 U/L |
| Cholesterol oxidase(CHO) | 20000U/L |
| N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5- dimethoxy-4-fluoroaniline, Sodium salt (F-DAOS) | 0.8mmol/L |

## Calibrator :

HDL-Cholesterol 50.6 mg/dl

**Storage:** 2ºC-8ºC

## Preparation of working solution:

Both the working reagent and the standard are supplied ready to use.

| | Blank | Calibrator | Test |
|---|---|---|---|
| Calibrator | -- | 2.4 µL | -- |
| Sample | -- | -- | 2.4 µL |
| Reagent 1 | 240 µL | 240µL | 240 µL |
| Mix, incubate 5min at 37°C,read absorbance $A_1$Then add: | | | |
| Reagent 2 | 60 µL | 60µL | 60µL |
| Mix, incubate 5 min at 37°C,read absorbance $A_2$. | | | |

## Procedure:

The sample and the working reagent are brought to room temperature prior to use. Working reagent should be gently shaken before each use.

3 test tubes are taken and labeled them as blank (B), Calibrator (C), Test (T). 240µL of working reagent 1 is added to 3 test tubes. 2.4µL of distilled water is added to test tube labeled 'B', 2.4µL of sample is added to test tube labeled 'T' and 2.4 µL of calibrator is added to test tube labeled 'C'. It is mixed and

incubate the tubes for 5 minutes at room temperature. Read the absorbance $A_1$ at 600/700nm, then 60µL of working reagent 2 is added to 3 test tubes. It is mixed and incubate the tubes for 5 minutes at room temperature. Read the absorbance $A_2$.

$\Delta A = (A_2 - A_1)$ sample or calibrator.

## Calculations:

$$\frac{\Delta A \text{Sample}}{\Delta A \text{ Calibrator}} \times \text{conc.Calib} = \text{Sample concentration(mg/dl)}$$

## Reference values:

Serum HDL-C $\geq$ 35mg/dl.

## CALCULATED PARAMETERS:

1. VERY LOW DENSITY LIPOPROTEIN-C = $\frac{\text{Triglycerides}}{5}$
2. LOW DENSITY LIPOPROTEIN-C = Total Cholesterol – HDL - $\frac{\text{Triglycerides}}{5}$

# RESULTS AND STATISTICAL ANALYSIS

## Table 1 Age distribution of the study population (n=80)

| Age group | Cases N (%) | Controls N (%) | Total N (%) |
|---|---|---|---|
| 21- 30 years | 0 (0) | 4 (13.3) | 4 (5) |
| 31 - 40 years | 6 (12) | 8 (26.7) | 14 (17.5) |
| 41 – 50 years | 2 (4) | 9 (30) | 11 (13.8.7) |
| 51 – 60 years | 16 (32) | 5 (16.7) | 21 (26.2) |
| 61 – 70 years | 20 (40) | 4 (13.3) | 24 (30) |
| 71 – 80 years | 6 (12) | 0 (0) | 6 (7.5) |
| Total | 50 (100) | 30 (100) | 80 (100) |

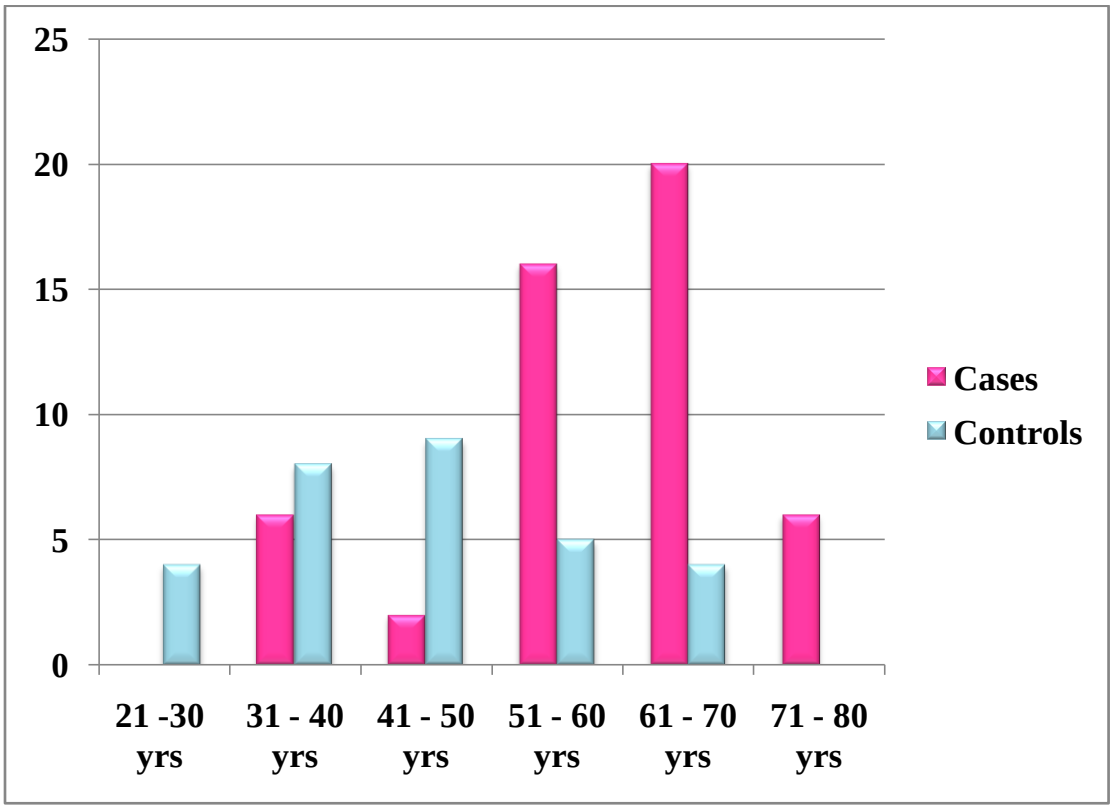
Mean age: 54.2 years

Standard deviation: 3.81 years

Minimum: 27 years

Maximum: 79 years

**Fig 1: Bar diagram showing Age distribution of the study population (n=80)**



## Table 2 Comparison of age among cases and controls (n=80)

## Student "t" test

| Group | Mean Age | Std. Deviation | Mean difference | p value | 95% confidence interval |
|---|---|---|---|---|---|
| Cases | 59.86 | 11.22 | 15.09 | 0.001 | 9.767 – 20.41 |
| Controls | 44.77 | 12.16 | | | |

**Comments:**

Subjects in the Cases group had a mean age of around 60 years and subjects in control group had a mean age of 45 years and this mean difference was statistically significant.

## Table 3 Gender distribution of the study population (n=80)

| Gender | Cases<br>N (%) | Controls<br>N (%) | Total<br>N (%) |
|---|---|---|---|
| Male | 32 (64) | 17 (56.7) | 49 (61.3) |
| Female | 18 (36) | 13 (43.3) | 31 (38.8) |
| Total | 50 (100) | 30 (100) | 80 (100) |

Chi square value: 0.425 p value: 0.515

## Comments:

Males and females were equally distributed in both cases and controls.

**Fig 2: Bar diagram showing sex distribution of the study population (n=80)**

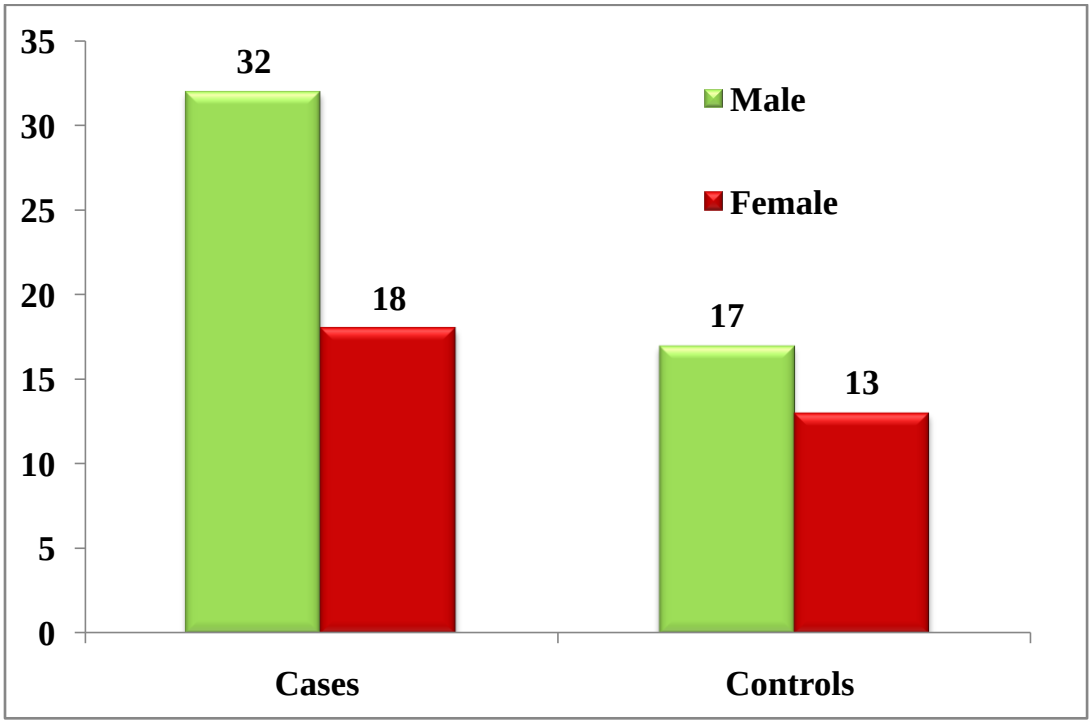


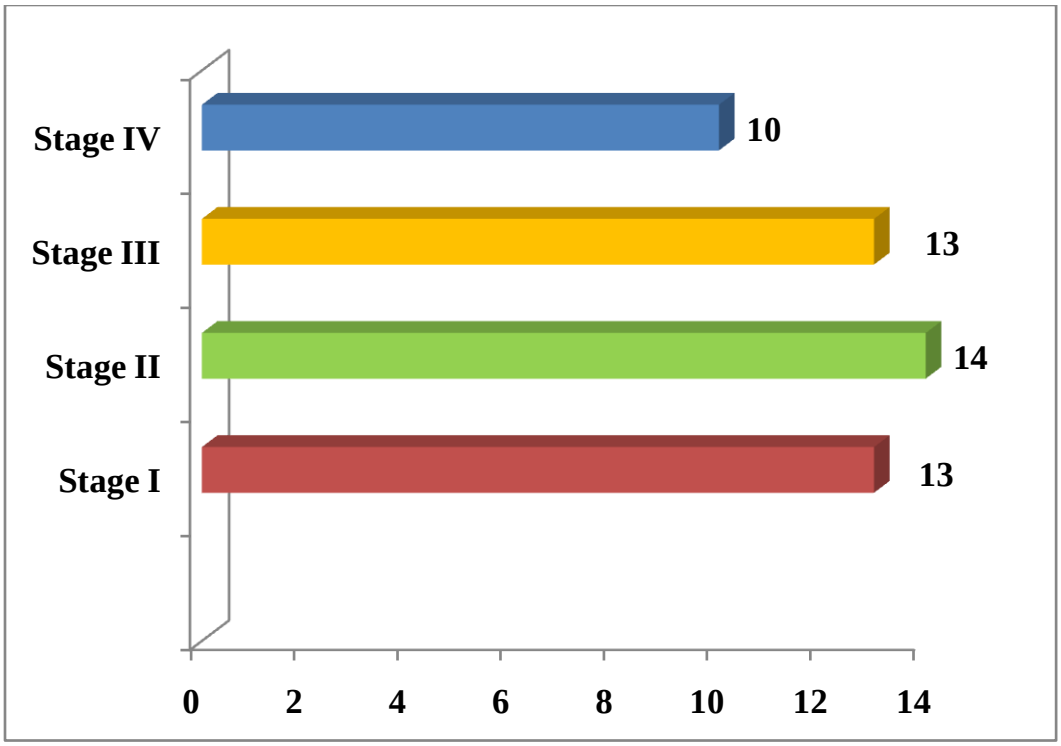
**Table 4 Distribution of the cases according to spirometry staging (n=50)**

| Spirometry stage | Frequency (N) | Percentage (%) |
|---|---|---|
| Stage I | 13 | 26 % |
| Stage II | 14 | 28 % |
| Stage III | 13 | 26 % |
| Stage IV | 10 | 20 % |
| Total | 50 | 100 % |

**Comments:**

Cases were distributed almost equally in all 4 stages of spirometry.

**Fig 3: Bar chart showing distribution of cases among stages of spirometry (n=50)**



## Table 5 Distribution of the cases according to BMI status (n=50)

| BMI classification* | Frequency (N) | Percentage (%) |
|---|---|---|
| Undernourished (<18.5) | 1 | 2 % |
| Normal (18.5 to 22.9) | 27 | 54 % |
| Overweight (23 to 24.9) | 11 | 22 % |
| Obese (>25 ) | 11 | 22 % |
| Total | 50 | 100 % |

*According to South Asian classification of obesity

Mean BMI: 23.28 $kg/m^2$

Standard deviation: 2.64 $kg/m^2$

Minimum BMI: 14 $kg/m^2$

Maximum BMI: 34 $kg/m^2$

## Comments:

About 44% of cases were either overweight or obese while 54% of cases had normal BMI.

**Fig 4:Pie chart showing distribution of cases according to BMI status (n=50)**

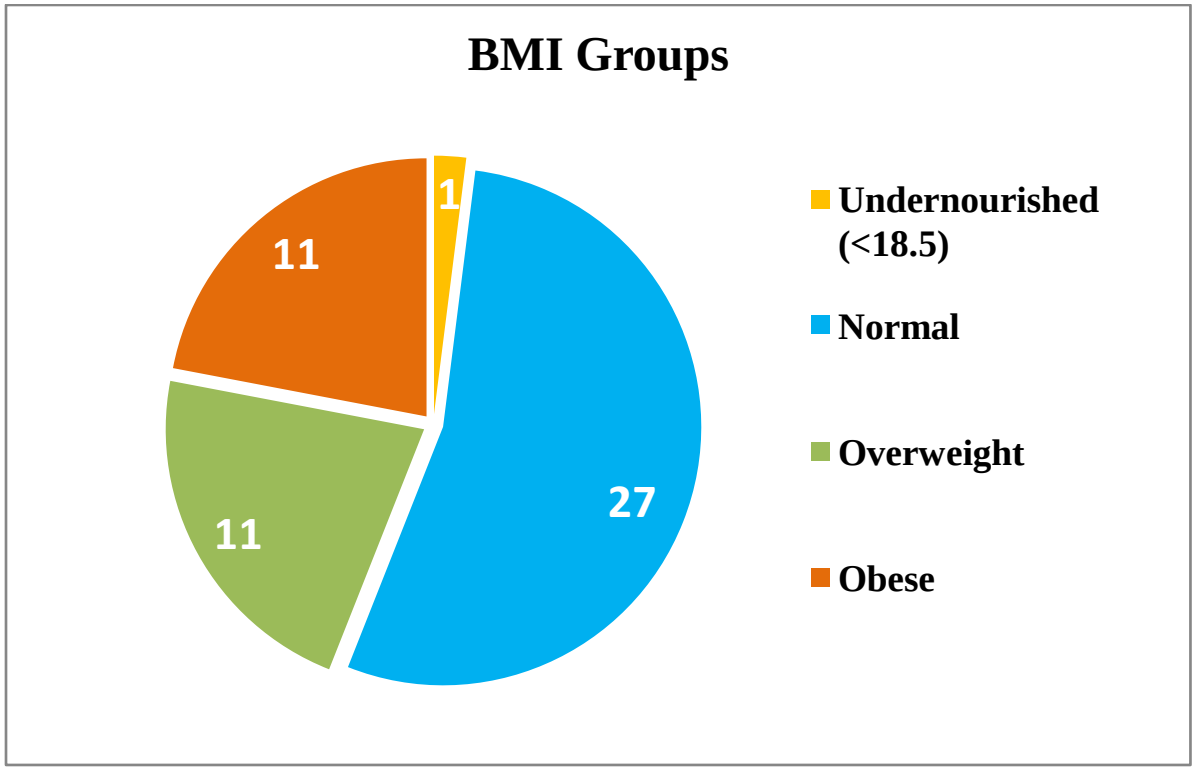


**Table 5 Distribution of the cases according to disease duration (n=50)**

| Disease duration | Frequency (N) | Percentage (%) |
|---|---|---|
| < 10 years | 10 | 20 % |
| 11 to 20 years | 32 | 64 % |
| >20 years | 8 | 16 % |
| Total | 50 | 100 % |

Mean disease duration: 15.08 years

Standard deviation: 5.2 years

Minimum: 6 years

Maximum: 28 years

**Comments:**

About 80% of cases had COPD for more than 10 years duration and 16% of cases had the disease for more than 20 years.

**Fig 5:Doughnut diagram showing distribution of cases according to duration of COPD (n=50)**

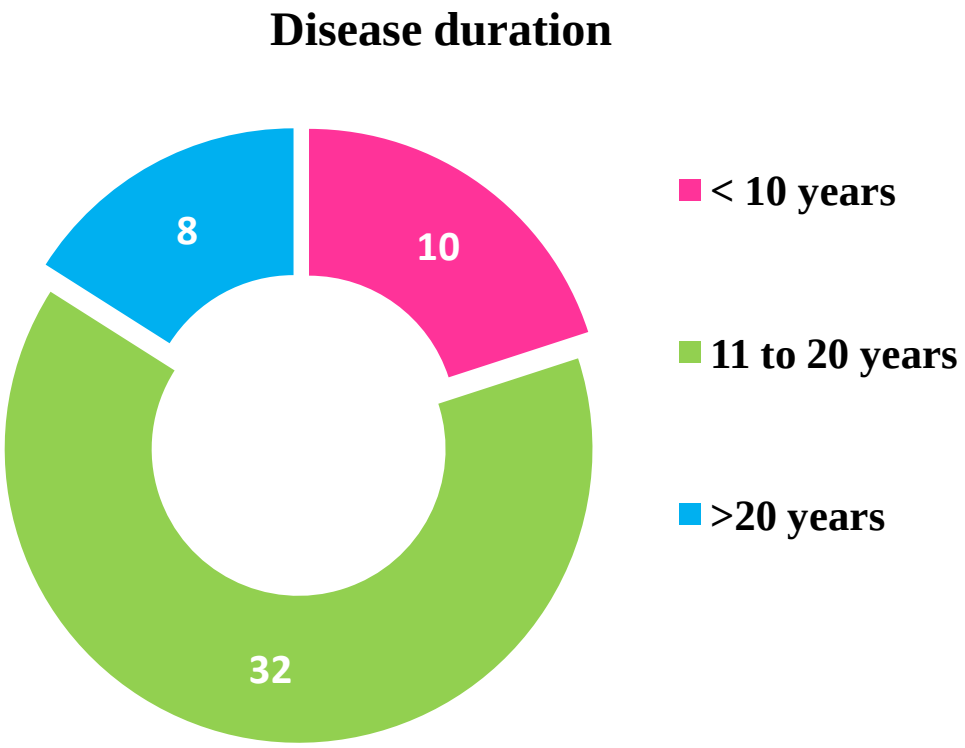


**Table 7 Distribution of the cases according to smoking status (n=50)**

| Smoking status | Frequency (N) | Percentage (%) |
|---|---|---|
| Non-smoker | 18 | 36 % |
| Ever smoker | 32 | 64 % |
| Total | 50 | 100 % |

Among Smokers:

Mean pack years: 25.71 years

Standard deviation: 7.53 years

Minimum: 12 years

Maximum: 39.5 years

**Comments:**

All the smokers among the cases were addicted for more than 10 years and the mean pack years of smoking (25) depicts the magnitude of the smoking.

**Fig 6: Pie diagram showing distribution of cases according to smoking status (n=50)**

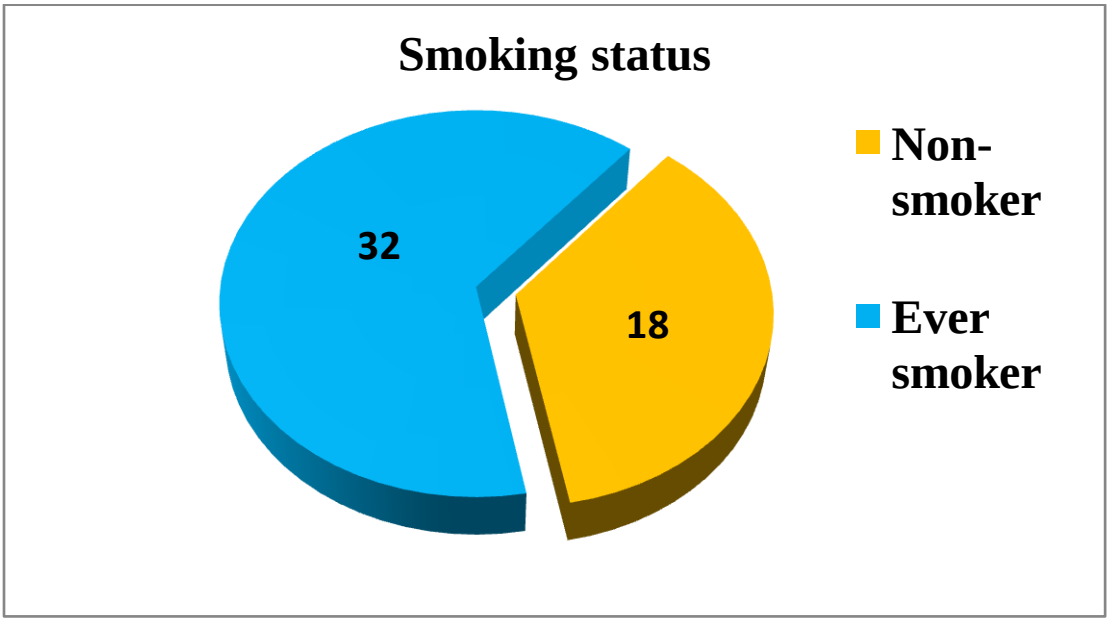


**Table 8 Comparison of HsCRP levels among cases and controls (n=80)**

**Student "t" test**

| Group | Mean HsCRP level (mg/L) | Std. Deviation | Mean difference | p value | 95% confidence interval |
|---|---|---|---|---|---|
| Cases | 12.66 | 13.92 | 9.16 | 0.001 | 4.066 – 14.254 |
| Controls | 3.50 | 1.57 | | | |

**Comments:**

Subjects in the Cases group had a mean HsCRP level of 12.66 mg/L which is roughly 9 units higher than the mean HsCRP level of control group (3.5 mg/L) and this mean difference was statistically significant.

**Fig 7: Bar diagram showing serum HsCRPlevel distribution of the study population (n=80)**

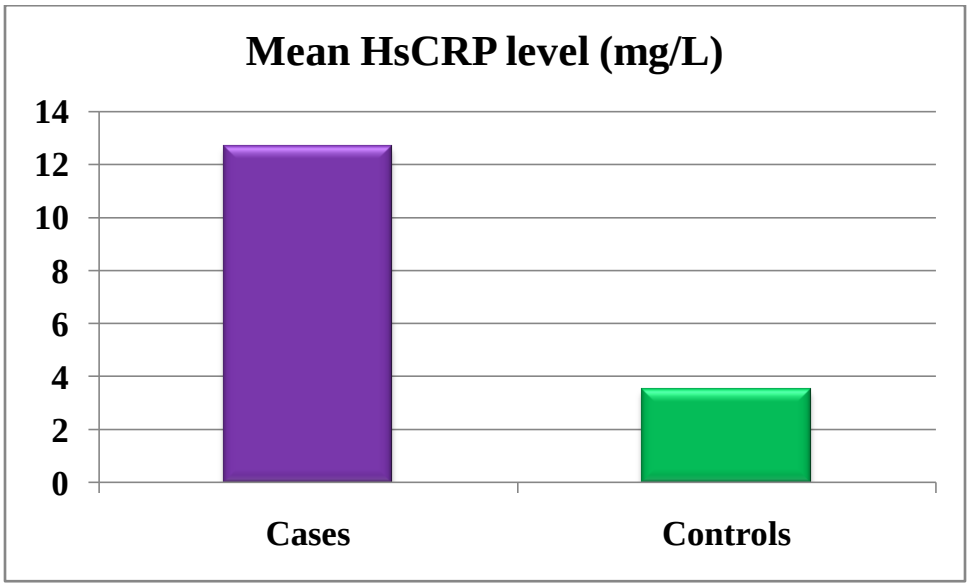


**Table 9 Distribution of the cases according to spirometry staging and HsCRP levels (n=50)**

| Spirometry staging | N | Mean HsCRP levels (mg/L) | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Stage I | 13 | 2.00 | .913 | 1.45 | 2.55 |
| Stage II | 14 | 4.36 | 2.499 | 2.91 | 5.80 |
| Stage III | 13 | 14.23 | 8.927 | 8.84 | 19.63 |
| Stage IV | 10 | 36.10 | 7.370 | 30.83 | 41.37 |
| Total | 50 | 12.66 | 13.928 | 8.70 | 16.62 |

ANOVA test was applied to test the difference in mean HsCRP levels between the groups followed by Bonferroni post-Hoc test for inter-group comparisons.

**ANOVA test**

| | |
|---|---|
| p value | <0.001 |
| F statistic | 79.528 |
| Degree of freedom | 3 |

## Bonferroni Post-Hoc test

| Dependent Variable (HsCRP levels) | Mean Difference | P value |
|---|---|---|
| Stage 1 Vs Stage 2 | -2.35 | 1.00 |
| Stage 1 Vs Stage 3 | -12.23 | <0.001* |
| Stage 1 Vs Stage 4 | -34.10 | <0.001* |
| Stage 2 Vs Stage 3 | -9.87 | <0.001* |
| Stage 2 Vs Stage 4 | -31.74 | <0.001* |
| Stage 3 Vs Stage 4 | -21.86 | <0.001* |

***significant at <0.05 level**

## Comments:

1) ANOVA test showed that there is a statistically significant difference in the mean HsCRP levels between the 4 groups.

2) Bonferroni test showed that the difference in mean HsCRP levels between Stage 1 Vs Stage 3, Stage 1 Vs Stage 4, Stage 2 Vs Stage 4 and Stage 3 Vs Stage 4 is statistically significant (all p values <0.05) and that the severity of disease increases with increase in mean HsCRP levels and vice versa.

**Table 10 Distribution of the cases according to spirometry staging and BMI levels (n=50)**

| Spirometry staging | N | Mean BMI | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Stage I | 13 | 20.154 | 1.4345 | 19.287 | 21.021 |
| Stage II | 14 | 21.571 | 3.8871 | 19.327 | 23.816 |
| Stage III | 13 | 24.446 | 3.9428 | 22.064 | 26.829 |
| Stage IV | 10 | 28.260 | 5.2502 | 24.504 | 32.016 |
| Total | 50 | 23.288 | 4.7150 | 21.948 | 24.628 |

ANOVA test was applied to test the difference in mean BMI levels between the groups followed by Bonferroni post-Hoc test for inter-group comparisons.

**ANOVA test**

| | |
|---|---|
| p value | <0.001 |
| F statistic | 10.139 |
| Degree of freedom | 3 |

## Bonferroni Post-Hoc test

| Dependent Variable (BMI levels) | Mean Difference | P value |
|---|---|---|
| Stage 1 Vs Stage 2 | -1.41 | 1.00 |
| Stage 1 Vs Stage 3 | -4.29 | 0.034* |
| Stage 1 Vs Stage 4 | -8.10 | <0.001* |
| Stage 2 Vs Stage 3 | -2.87 | 0.324 |
| Stage 2 Vs Stage 4 | -6.68 | 0.001* |
| Stage 3 Vs Stage 4 | -3.81 | 0.123 |

***significant at <0.05 level**

**Comments:**

1) ANOVA test showed that there is a statistically significant difference in the mean BMI levels between three groups.

2) Bonferroni test showed that the difference in mean BMI levels between Stage 1 Vs Stage 3, Stage 1 Vs Stage 4 and Stage 2 Vs Stage 4 is statistically significant (all p values <0.05) and that the severity of disease increases with increase mean BMI levels and vice versa.

## Table 11 Distribution of the cases according to spirometry staging and duration of COPD (n=50)

| Spirometry staging | N | Mean duration of COPD in years | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Stage I | 13 | 14.54 | 5.840 | 11.01 | 18.07 |
| Stage II | 14 | 14.29 | 3.970 | 11.99 | 16.58 |
| Stage III | 13 | 14.69 | 5.170 | 11.57 | 17.82 |
| Stage IV | 10 | 17.40 | 6.041 | 13.08 | 21.72 |
| Total | 50 | 15.08 | 5.213 | 13.60 | 16.56 |

ANOVA test was applied to test the difference in mean duration of COPD in years between the groups.

**ANOVA test**

| | |
|---|---|
| p value | 0.484 |
| F statistic | 0.831 |
| Degree of freedom | 3 |

**Comments:**

There was no statistically significant association between duration of COPD and spirometry staging.

**Table 12 Distribution of the cases according to spirometry staging and smoking as categorical variable (n=50)**

| Spirometry stage | Smoker N (%) | Non-smokers N (%) | Total N (%) |
|---|---|---|---|
| Stage 1 | 9 (28.1) | 4 (22.2) | 13 (26) |
| Stage 2 | 7 (21.9) | 7 (38.9) | 14 (28) |
| Stage 3 | 7 (21.9) | 6 (33.3) | 13 (26) |
| Stage 4 | 9 (28.1) | 1 (5.6) | 10 (20) |
| Total | 32 (100) | 18 (100) | 50 (100) |

Chi-square value: 4.861 p Value: 0.182

**Comments:**

There was no statistically significant association between smoking status and spirometry staging of COPD when smoking is taken as a binomial categorical variable.

**Table 13 Distribution of the cases according to spirometry staging and smoking as continuous variable (in pack-years) (n=50)**

| Spirometry staging | N | Mean smoking pack-years | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Stage I | 13 | 11.81 | 9.2478 | 6.219 | 17.396 |
| Stage II | 14 | 12.11 | 12.6691 | 4.792 | 19.422 |
| Stage III | 13 | 15.42 | 15.1215 | 6.285 | 24.561 |
| Stage IV | 10 | 29.95 | 11.2779 | 21.882 | 38.018 |
| Total | 50 | 16.46 | 13.8372 | 12.528 | 20.392 |

ANOVA test was applied to test the difference in mean smoking pack-years between the groups followed by Bonferroni post-Hoc test for inter-group comparisons.

**ANOVA test**

| | |
|---|---|
| p value | 0.003 |
| F statistic | 5.213 |
| Degree of freedom | 3 |

## Bonferroni Post-Hoc test

| Dependent Variable (Smoking pack-years) | Mean Difference | P value |
|---|---|---|
| Stage 1 Vs Stage 2 | -0.299 | 1.00 |
| Stage 1 Vs Stage 3 | -3.61 | 1.00 |
| Stage 1 Vs Stage 4 | -18.14 | 0.006* |
| Stage 2 Vs Stage 3 | -3.31 | 1.00 |
| Stage 2 Vs Stage 4 | -17.84 | 0.006* |
| Stage 3 Vs Stage 4 | -14.52 | 0.045* |

***significant at <0.05 level**

## Comments:

1) ANOVA test showed that there is a statistically significant difference in the mean smoking pack-years between three groups.

2) Bonferroni test showed that the difference in mean BMI levels between Stage 1 Vs Stage 4, Stage 2 Vs Stage 4 and Stage 3 Vs Stage 4 is statistically significant (all p values <0.05) and that the severity of disease increases with increase mean smoking pack-years but significantly increased in stage 4 and vice versa.

**Fig 8 : Showing comparison of various parameters according to stages of spirometry among cases (n=50)**

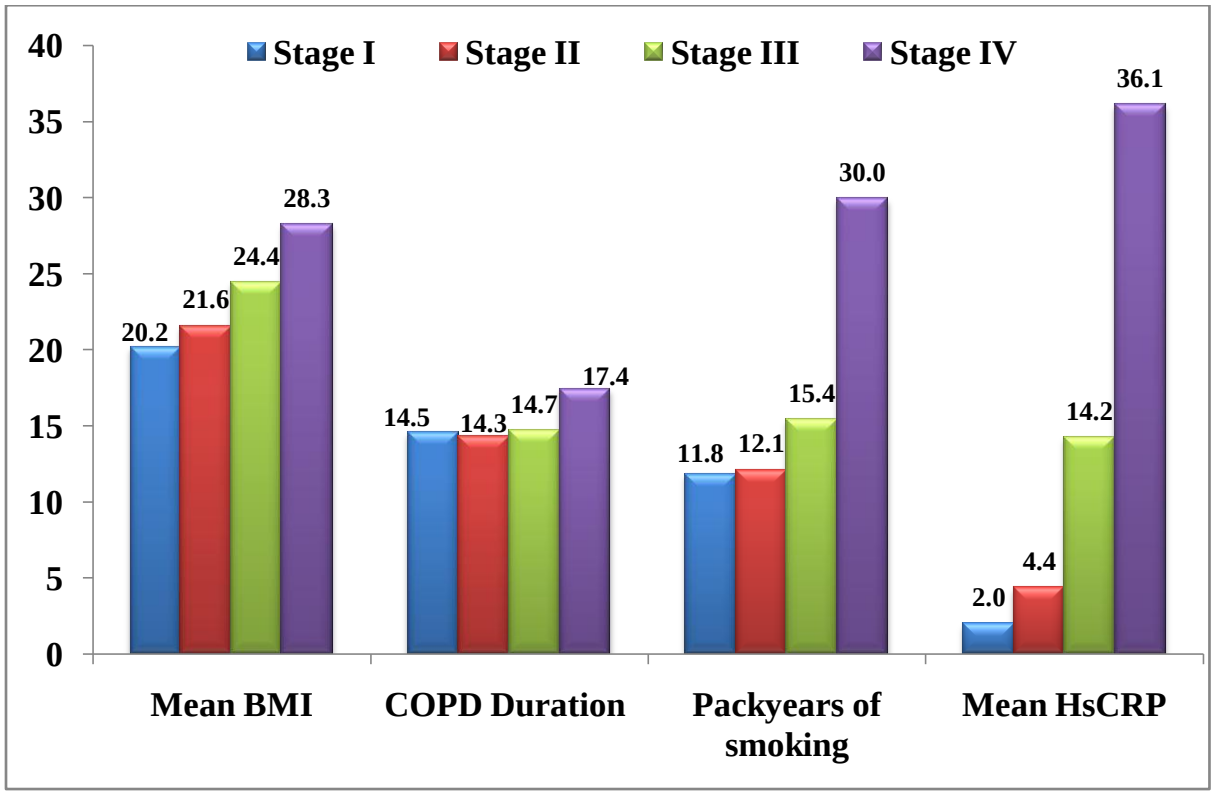


**Table 14 Distribution of the cases according to Duration of COPD and BMI levels (n=50)**

| BMI | Duration of COPD | | | Total |
|---|---|---|---|---|
| | <10 years | 11 to 20 years | >20 years | |
| <18.5 | 0 (0%) | 1 (2%) | 0 (0%) | 1 (2%) |
| 18.5 - 22.9 | 5 (10%) | 18 (36%) | 4 (8%) | 27 (54%) |
| 23 - 24.9 | 2 (4%) | 6 (12%) | 3 (6%) | 11 (22%) |
| >25 | 3 (6%) | 7 (14%) | 1 (2%) | 11 (22%) |
| Total | 10 (20%) | 32 (64%) | 8 (16%) | 50 (100%) |

Chi square value: 2.311 p value: 0.889

**Comments**:

There was no statistically significant association between BMI levels and duration of COPD.

**Table 15 Comparison of smoking status and duration of COPD among cases (n=50)**

**Student "t" test**

| Group (N) | Mean duration of COPD | Std. Deviation | Mean difference | p value | 95% confidence interval |
|---|---|---|---|---|---|
| Smokers (32) | 16.25 | 5.22 | 3.25 | 0.033* | 0.276 – 6.224 |
| Non-smokers (18) | 13 | 4.62 | | | |

## Comments:

Smokers had a mean duration of COPD of around 16 years while cases not exposed to smoking had a mean duration of 13 years and this mean difference was statistically significant. Hence, the disease either sets early or persists longer in smokers.

**Table 16 Comparison of Lipoprotein(a) levels among cases and controls (n=80)**

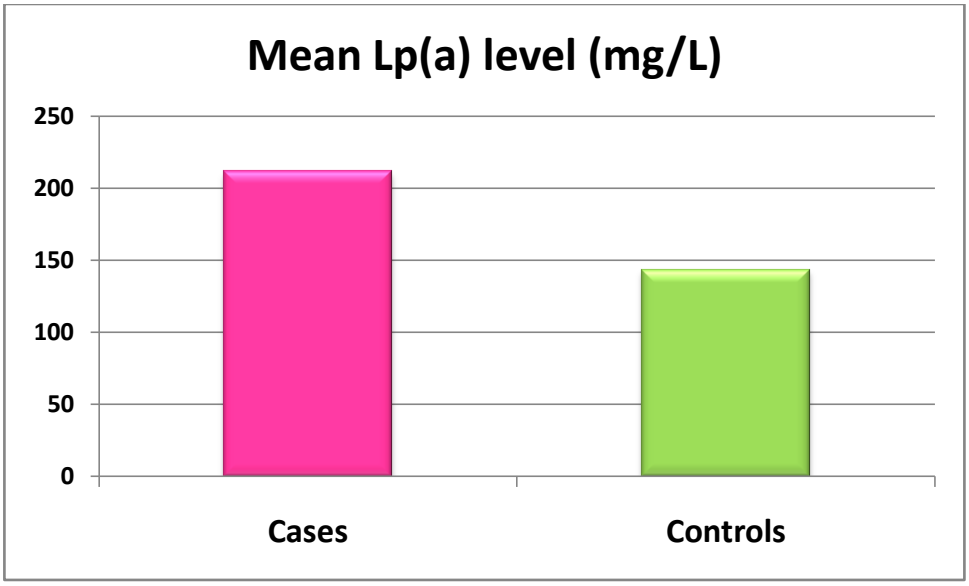
**Student "t" test**

| Group | Mean LP(a) level (mg/L) | Std. Deviation | Mean difference | p value | 95% confidence interval |
|---|---|---|---|---|---|
| Cases | 211.68 | 143.00 | 68.447 | 0.002 | 25.69 - 111.19 |
| Controls | 143.23 | 37.84 | | | |

**Comments:**

Subjects in the Cases group had a mean LP(a) level of 211 mg/L which is roughly 68 units higher than the mean LP(a) level of control group (143 mg/L) and this mean difference was statistically significant.

**Fig 9: Bar diagram showing serum Lipoprotein(a) level distribution of the study population (n=80)**



## Table 17 Distribution of the cases according to spirometry staging and Lipoprotein (a) levels (n=50)

| Spirometry staging | N | Mean Lp(a) levels (mg/L) | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Stage I | 13 | 220.46 | 115.784 | 150.49 | 290.43 |
| Stage II | 14 | 212.29 | 142.973 | 129.74 | 294.84 |
| Stage III | 13 | 183.38 | 199.074 | 63.09 | 303.68 |
| Stage IV | 10 | 236.20 | 95.705 | 167.74 | 304.66 |
| Total | 50 | 211.68 | 143.003 | 171.04 | 252.32 |

ANOVA test was applied to test the difference in mean Lp(a) levels between the groups followed by Bonferroni post-Hoc test for inter-group comparisons.

**ANOVA test**

| | |
|---|---|
| p value | 0.846 |
| F statistic | 0.271 |
| Degree of freedom | 3 |

**Comments:**

ANOVA test showed that there is no statistically significant association between mean Lipoprotein(a) levels and the spirometry staging.

**Fig 10 : Showing comparison of mean serum lipoprotein (a) levels according to stages of spirometry among cases (n=50)**

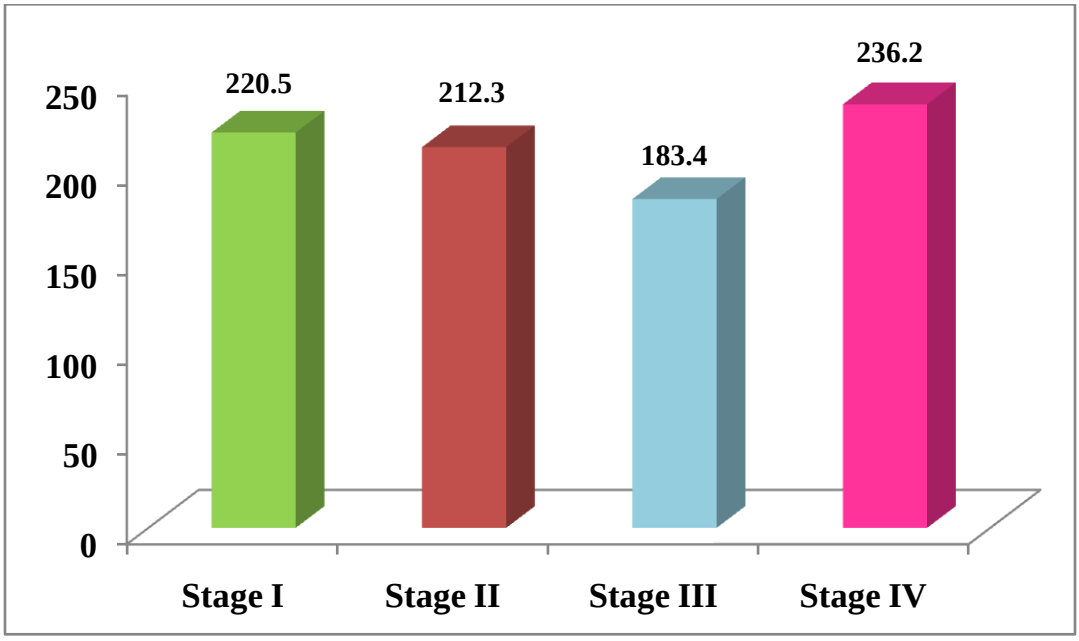


**Table 18 Comparison of lipid parameters among cases and controls (n=80)**

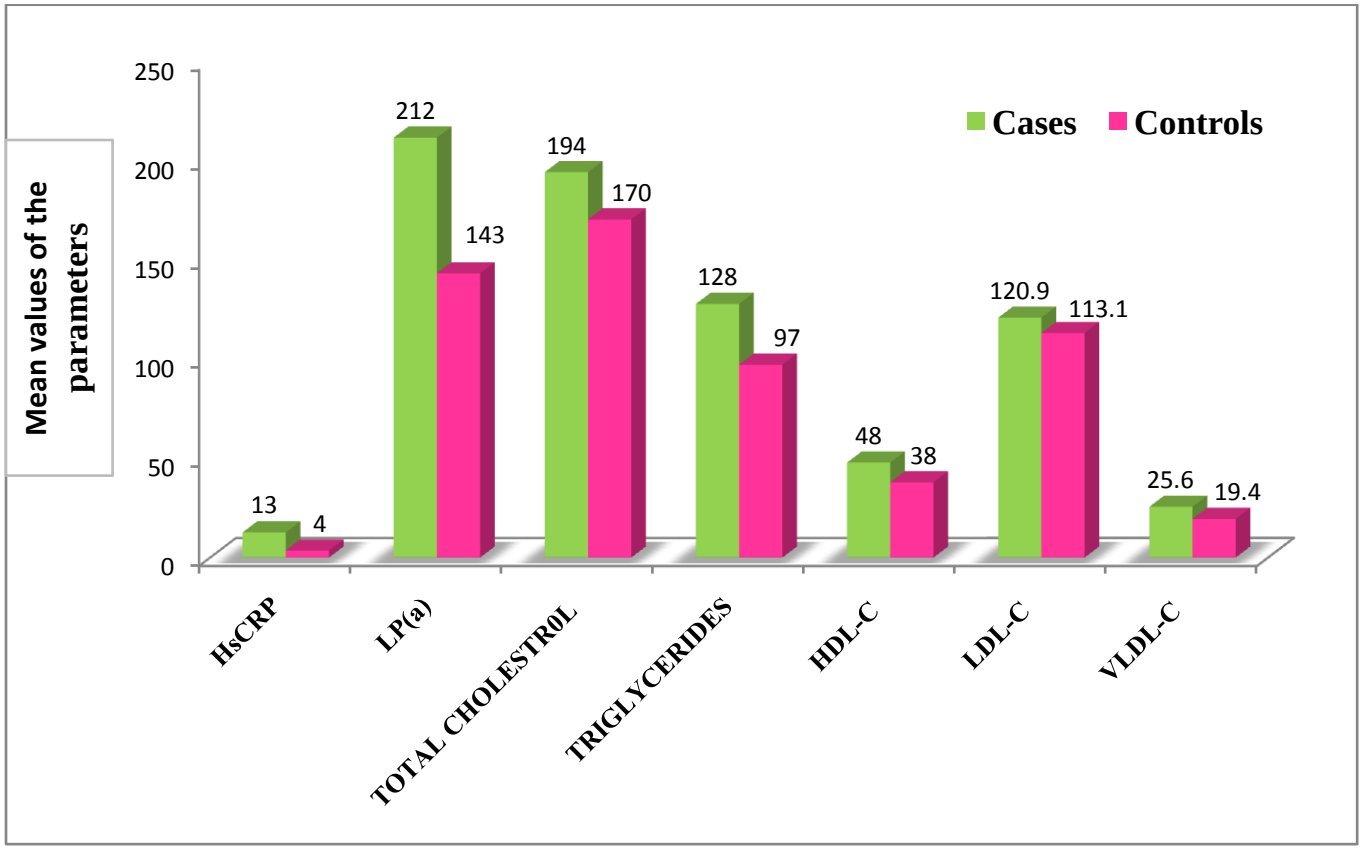
**Student "t" test**

| Lipid parameters | | Cases (n=50) | Controls (n=30) | Mean difference (Cases - Controls) | Student t test p value |
|---|---|---|---|---|---|
| Total Cholesterol (mg/dl) | Mean levels | 194.30 | 170.43 | 23.867 | 0.001 |
| | Standard deviation | 42.75 | 19.63 | | |
| Triglycerides (mg/dl) | Mean levels | 127.88 | 97.17 | 30.713 | <0.001 |
| | Standard deviation | 44.39 | 14.83 | | |
| HDL cholesterol (mg/dl) | Mean levels | 47.88 | 37.87 | 10.013 | <0.001 |
| | Standard deviation | 12.50 | 5.99 | | |
| LDL cholesterol (mg/dl) | Mean levels | 120.88 | 113.13 | 7.754 | 0.177 |
| | Standard deviation | 33.53 | 17.27 | | |
| VLDL cholesterol (mg/dl) | Mean levels | 25.57 | 19.43 | 6.138 | <0.001 |
| | Standard deviation | 8.87 | 2.96 | | |

**Comments:**

Cases had high levels of all the lipid parameters than controls and this difference in the mean levels of the above mentioned parameters were also statistically significant except for LDL cholesterol levels.

**Fig 11: Clustered Bar chart comparing the mean serum levels of various parameters among cases and controls (N=80)**



## Table 19: Correlation matrix between BMI , serum markers and Lipid parameters (n=50)

| | BMI | Hs-CRP mg/L | Lp(a) mg/L | Total Cholesterol mg/dl | Triglycerides mg/dl | HDL-C mg/dl | LDL-C mg/dl | VLDL-C mg/dl |
|---|---|---|---|---|---|---|---|---|
| BMI | 1 | 0.640** | -0.40 | -0.191 | -0.224 | -0.311* | -0.068 | -0.224 |

***p value<0.05**

**Comments :**

1) There was a statistically significant positive linear correlation between BMI and serum Hs-CRP levels. i.e the increase in BMI also had a corresponding increase in serum Hs-CRP levels in the same direction.
2) There was a statistically significant negative linear correlation between BMI and serum HDL levels. i.e the increase in BMI also had a corresponding decrease in serum HDL levels in the opposite direction.

## Table 20: Correlation matrix between disease duration, serum markers and Lipid parameters (n=50)

| | Disease duration | Hs-CRP mg/L | Lp(a) mg/L | Total Cholesterol mg/dl | Triglycerides mg/dl | HDL-C mg/dl | LDL-C mg/dl | VLDL-C mg/dl |
|---|---|---|---|---|---|---|---|---|
| Disease duration | 1 | 0.099 | 0.098 | 0.073 | 0.013 | 0.0111 | 0.047 | 0.013 |

***p value<0.05**

## Comments:

There was no statistically significant correlation between disease duration and Hs-CRP, Lp(a) and Lipid profile.

## Table 21 Multivariate Logistic regression model for COPD with predictors (n=80)

| Independent variable | Dependent variable (COPD) | | p value |
|---|---|---|---|
| | Adjusted Odds ratio (AOR) | 95% confidence interval | |
| Age in years | 1.144 | 1.067 – 1.226 | ***<0.001**** |
| Sex<br>Female<br>Male | <br>2.811<br>1 | <br>0.796 – 18.23<br>- | ***0.094*** |
| Serum HsCRP levels | 1.259 | 1.03 – 1.52 | ***0.019*** |
| Serum Lp (a) levels | 1.007 | 0.99 – 1.01 | 0.093 |

**Nagelkerke's pseudo R square-62.3%**

**Comments:**

1. For every unit increase in age, the risk of COPD increased by 14% even after adjusting for other variables in the model and this odds ratio was statistically significant.
2. For every unit increase in Serum HsCRP levels, the risk of COPD increased by 25% after adjusting for other variables in the model and this odds ratio was statistically significant.
3. For every unit increase in Serum Lp(a) levels, the risk of COPD increased by 7% after adjusting for other variables in the model and females had a relatively higher risk of being in cases group with COPD than controls but these odds ratios were not statistically significant
4. About 62.5% of variability in the occurrence of COPD can be explained by these 4 variables included in the model as given by Nagelkerke's pseudo R square.

## Table 22: Correlation matrix between smoking, serum markers and lipid parameters (n=50)

| | **SMOKING** | **HsCRP (mg/L)** | **LP(a) (mg/L)** | **TOTAL CHOLESTR0L (mg/dl)** | **TRIGLYCERIDES (mg/dl)** | **HDL-C (mg/dl)** | **LDL-C (mg/dl)** | **VLDL-C (mg/dl)** |
|---|---|---|---|---|---|---|---|---|
| SMOKING | 1 | | | | | | | |
| HsCRP (mg/L) | 0.405* | 1 | | | | | | |
| LP(a) (mg/L) | 0.110 | 0.045 | 1 | | | | | |
| TOTAL CHOLESTR0L (mg/dl) | 0.309 | -0.257 | 0.347* | 1 | | | | |
| TRIGLYCERIDES (mg/dl) | -0.053 | -0.118 | 0.127 | 0.316* | 1 | | | |
| HDL-C (mg/dl) | -0.286* | -0.376* | 0.105 | 0.661* | 0.041 | 1 | | |
| LDL-C (mg/dl) | -0.275 | -0.156 | 0.368* | 0.945* | 0.125 | 0.459 | 1 | |
| VLDL-C (mg/dl) | -0.052 | -0.118 | 0.128 | 0.316* | 0.31* | 0.041 | 0.125 | 1 |

***p value<0.05**

**Comments:**

1) There was a statistically significant positive linear correlation between smoking and serum HsCRPlevels.i.e. the increase in pack years of smoking also had a corresponding increase in serum HsCRP levels in the same direction.
2) There was a statistically significant negative linear correlation between smoking and serum HDL levels.i.e. the increase in pack years of smoking also had a corresponding decrease in serum HDL levels in the opposite direction.
3) There was a statistically significant negative linear correlation between serum HsCRP levels and serum HDL levels .i.e. the increase in serum HsCRP levels also had a corresponding decrease in serum HDL levels in the opposite direction.
4) There was a statistically significant positive linear correlation between serum lipoprotein (a) levels and serum total cholesterol , LDL cholesterol levels.i.e. the increase in serum lipoprotein (a) levels also had a corresponding increase serum total cholesterol , LDL cholesterol levels in the same direction.
5) There was a statistically significant positive linear correlation between serum total cholesterol and all other lipid parameters as expected.
6) There was a statistically significant positive linear correlation between serum triglyceride levels and serum VLDL cholesterol levels.i.e. the increase in serum triglyceride levels also had a corresponding increase in serum VLDL cholesterol levels in the same direction.

# DISCUSSION

Chronic obstructive pulmonary disease is a major public health concern and is a leading cause of morbidity and mortality. The reliance upon expert opinion to develop the various worldwide COPD guidelines and the variation in the guidelines also likely reflect the fact that COPD is a heterogenous disease. The biomarkers to determine the severity of COPD are necessary in the control of disease prognosis.

High Sensitivity C-Reactive Protein is one of the recently identified valid biomarker of low-grade systemic inflammation.[59]

In the present study, the mean Hs-CRP level in the case group was (12.66±13.92) significantly higher than in the control group (3.50±1.57) with p value 0.001. These results were supported by studies of Lisatileman , Lena ginder et al.,[60] and Sanjamarevic et al.,[61]both were proposed that Hs-CRP levels are significantly elevated in COPD patients than the control group.

In the present study, there was a significant increase in Hs-CRP in stage III and stage IV COPD compared to stage I or stage II. The severity of disease increases with increase in mean Hs-CRP levels. These results were supported by studies of Tahia H saleem ,Manal A M Mandour et al.,[59] and SAalavi et al.,[62] both were proposed that Hs-CRP level was significantly correlated to stage of disease (as gold criteria).

In the present study, there was a significant increase in Hs-CRP level in smokers with COPD than in non smokers with COPD and also the number of pack-years was positively correlated with Hs-CRP levels. These results were supported by studies of SAalavi et al.,[62]and Yannick M.T.A. et al.,[63]and Reshu Agarwal et al.,[64]were proposed that Hs-CRP level was significantly higher in smokers as compared to non smokers.

In the present study, there was a significant increase in BMI level also had a corresponding increase in serum Hs-CRP levels. These results were supported by studies of SAalavi et al.,[62] Breyer MK et al.,[65] and PO Bridevaux et al.,[66] were proposed that Hs-CRP level was significantly higher in obese COPD patients as compared to nonobese patients.

In the present study, there was a no correlation between the disease duration and Hs-CRP levels. These results were supported by study of Daianastolz et al.,[67] who concluded the same in a group of 100 patients for a follow up period of longer time >1yr.

In the present study, there was a significant negative correlation between serum Hs-CRP levels and serum HDL levels. The increase in serum Hs-CRP levels also had a corresponding decrease in serum HDL levels.

In the present study, there was a significant increase in severity of the disease also had a corresponding increase in mean BMI levels.

In the present study, there was a significant increase in severity of the disease also had a corresponding increase in mean smoking pack-years and also significantly increased in stage IV.

In the present study, there was a significant increase in age ,the risk of COPD also increased.

In the present study, there was no significant association between duration of COPD and spirometry staging.

In the present study, there was no significant association between BMI levels and duration of COPD.

COPD is a chronic inflammatory disease of the lung, that is known to have systemic features, among which is an increased risk of cardiovascular disease. Cardiovascular diseases are the most potent killers particularly so in the advanced countries of the world. Cardiovascular disease is a leading cause of death in patients with COPD.[70]

Lipoprotein (a) has emerged as a powerful genetic risk factor for coronary artery disease.[66]

In the present study, there was a significant increase in the mean Lipoprotein(a) level in the case group was (211.68±143.00) which was significantly higher than in the control group (143.23±37.84) with p value 0.002.

In the present study, there was a significant positive correlation between serum lipoprotein(a) levels and serum total cholesterol , LDL cholesterol levels. The increase in serum lipoprotein(a) levels also had a corresponding increase serum total cholesterol and LDL cholesterol levels.

In the present study, there was no significant association between mean Lipoprotein(a) levels and the spirometry staging, BMI, smoking, disease duration.

Lipid parameter abnormalities are a highly important. Its alteration in lipid metabolism increases cardiovascular risk, and so the mortality and morbidity[71].

In the present study, the mean levels of the all lipid parameters of case group had high level than the control group .P values of all lipid parameters were significant except LDL cholesterol level.

In the present study, there was a significant negative correlation between smoking and serum HDL levels. The increase in pack years of smoking also had a corresponding decrease in serum HDL levels.

In the present study, there was a significant negative correlation between BMI levels and serum HDL levels. The increase in BMI levels also had a corresponding decrease in serum HDL levels.

In the present study, there was a significant positive correlation between serum total cholesterol and all other lipid parameters.

In the present study, there was a significant positive correlation between serum triglyceride levels and serum VLDL cholesterol levels. The increase in serum triglyceride levels also had a corresponding increase in serum VLDL cholesterol levels.

In the present study, there was no significant association between Total cholesterol, Triglycerides, LDL, VLDL and BMI levels, smoking.

In the present study, there was no significant association between all lipid parameters and disease duration.

# CONCLUSION

The present study confirms that, circulating Hs-CRP levels are higher in COPD patients and may thus be regarded as a valid biomarker of low-grade systemic inflammation.

The levels of Hs-CRP are significantly elevated in smokers with COPD, obese COPD patients and also correlates with the severity of the disease.

This novel bio-marker is a valuable marker in staging and a predictor of COPD progression and management decision including administration of specific preventive and therapeutic strategies, potentially resulting in fewer morbidity and mortality.

The present study confirms that, circulating Lipoprotein(a) levels are higher in COPD patients may thus be regarded as a valid biomarker of atherothrombotic acute events. The increase in serum lipoprotein(a) levels also had a corresponding increase serum total cholesterol and LDL cholesterol levels.

High serum Lipoprotein(a) level observed in this study with high frequency of atherosclerotic disease such as coronary artery disease and cerebrovascular disease described in COPD patients.

This novel bio-marker is a valuable marker for predictor of atherothrombotic risk in COPD patients and management decision including administration of specific preventive and therapeutic strategies, potentially resulting in fewer morbidity and mortality.

In the present study, there is no association between mean Lipoprotein(a) levels and the spirometry staging, BMI, smoking, disease duration.

# SUMMARY

Serum High Sensitivity C-Reactive Protein is a marker of systemic inflammation and functional disability in COPD patients.

Increasing levels of Hs-CRP may means the infectious state in human body and can guide the clinical treatment of COPD .

Serum Hs-CRP may be used as a simple auxiliary marker in staging and determining the prognosis of COPD for early intervention.

Serum Lipoprotein(a) is a marker of atherosclerotic disease such as coronary artery disease and cerebrovascular diseas in COPD patients.

Serum Lipoprotein(a) may be used as a simple auxiliary marker in determining the risk of atherosclerotic disease in COPD patients for early intervention.

## MASTER CHART - I CONTROL GROUP

| S.NO | AGE (years) | SEX | BMI kg/m2 | HsCRP (mg/L) | LP(a) (mg/L) | TOTAL CHOLESTROL (mg/dl) | TRIGLYCERIDES (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | VLDL-C (mg/dl) | GLUCOSE (mg/dl) | UREA (mg/dl) | CREATININE (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 33 | MALE | 21.5 | 5 | 157 | 157 | 76 | 35 | 106.8 | 15.2 | 85 | 28 | 0.8 |
| 2 | 52 | MALE | 34.8 | 6 | 146 | 175 | 91 | 42 | 114.8 | 18.2 | 97 | 19 | 0.6 |
| 3 | 36 | FEMALE | 34.05 | 3 | 116 | 139 | 102 | 31 | 87.6 | 20.4 | 79 | 28 | 0.9 |
| 4 | 54 | MALE | 22.6 | 5 | 112 | 140 | 109 | 41 | 77.2 | 21.8 | 92 | 28 | 0.7 |
| 5 | 39 | MALE | 23.4 | 3 | 185 | 193 | 114 | 31 | 139.2 | 22.8 | 91 | 17 | 1.1 |
| 6 | 47 | MALE | 27 | 3 | 185 | 149 | 76 | 31 | 102.8 | 15.2 | 87 | 38 | 0.7 |
| 7 | 34 | MALE | 21.5 | 6 | 92 | 141 | 92 | 41 | 81.6 | 18.4 | 101 | 25 | 0.9 |
| 8 | 43 | FEMALE | 27.8 | 4 | 171 | 191 | 86 | 49 | 124.8 | 17.2 | 108 | 33 | 1.2 |
| 9 | 42 | MALE | 30.4 | 5 | 168 | 185 | 103 | 37 | 127.4 | 20.6 | 98 | 29 | 0.8 |
| 10 | 29 | FEMALE | 34.2 | 6 | 96 | 180 | 101 | 39 | 120.8 | 20.2 | 84 | 20 | 0.9 |
| 11 | 31 | FEMALE | 30.2 | 3 | 175 | 167 | 115 | 36 | 108 | 23 | 89 | 28 | 0.7 |
| 12 | 31 | FEMALE | 24.2 | 3 | 98 | 191 | 107 | 37 | 132.6 | 21.4 | 94 | 34 | 1.1 |
| 13 | 51 | FEMALE | 34.2 | 3 | 161 | 147 | 90 | 35 | 94 | 18 | 104 | 20 | 0.9 |
| 14 | 44 | MALE | 21.2 | 2 | 190 | 167 | 94 | 32 | 116.2 | 18.8 | 80 | 28 | 0.8 |
| 15 | 29 | FEMALE | 21.2 | 1 | 190 | 159 | 101 | 40 | 98.8 | 20.2 | 90 | 38 | 1.2 |
| 16 | 48 | FEMALE | 21.2 | 1 | 105 | 195 | 110 | 53 | 120 | 22 | 82 | 25 | 0.6 |
| 17 | 27 | MALE | 30.2 | 1 | 81 | 135 | 94 | 32 | 84.2 | 18.8 | 88 | 28 | 0.7 |
| 18 | 70 | MALE | 21.7 | 3 | 157 | 161 | 86 | 31 | 112.8 | 17.2 | 85 | 28 | 0.8 |
| 19 | 47 | FEMALE | 20 | 5 | 105 | 208 | 111 | 51 | 134.8 | 22.2 | 90 | 28 | 0.9 |
| 20 | 48 | MALE | 21.3 | 2 | 157 | 183 | 117 | 46 | 113.6 | 23.4 | 92 | 22 | 0.7 |
| 21 | 67 | MALE | 25.6 | 4 | 54 | 155 | 78 | 35 | 104.4 | 15.6 | 92 | 32 | 1.1 |
| 22 | 57 | FEMALE | 22.6 | 5 | 185 | 192 | 87 | 44 | 130.6 | 17.4 | 89 | 28 | 0.7 |
| 23 | 30 | FEMALE | 30.4 | 5 | 110 | 173 | 81 | 34 | 122.8 | 16.2 | 100 | 29 | 0.9 |
| 24 | 58 | MALE | 20.8 | 1 | 130 | 165 | 77 | 36 | 113.6 | 15.4 | 83 | 35 | 0.8 |
| 25 | 40 | FEMALE | 20.8 | 3 | 155 | 160 | 135 | 41 | 92 | 27 | 82 | 31 | 0.8 |
| 26 | 65 | MALE | 26.9 | 4 | 147 | 187 | 105 | 34 | 132 | 21 | 102 | 38 | 0.7 |
| 27 | 35 | FEMALE | 20.5 | 5 | 135 | 194 | 102 | 35 | 138.6 | 20.4 | 101 | 25 | 0.8 |
| 28 | 62 | MALE | 32 | 2 | 175 | 171 | 76 | 39 | 116.8 | 15.2 | 99 | 29 | 1.2 |
| 29 | 49 | MALE | 23.7 | 4 | 187 | 184 | 112 | 35 | 126.6 | 22.4 | 0.7 | 18 | 0.7 |
| 30 | 45 | MALE | 30.2 | 2 | 172 | 169 | 87 | 33 | 118.6 | 17.4 | 84 | 38 | 0.6 |

**MASTER CHART - II CONTROL GROUP**

| S.NO | AGE (years) | SEX | BMI kg/m2 | HsCRP (mg/L) | LP(a) (mg/L) | TOTAL CHOLESTROL (mg/dl) | TRIGLYCERIDES (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | VLDL-C (mg/dl) | GLUCOSE (mg/dl) | UREA (mg/dl) | CREATININE (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 33 | MALE | 21.5 | 5 | 157 | 157 | 76 | 35 | 106.8 | 15.2 | 85 | 28 | 0.8 |
| 2 | 52 | MALE | 34.8 | 6 | 146 | 175 | 91 | 42 | 114.8 | 18.2 | 97 | 19 | 0.6 |
| 3 | 36 | FEMALE | 34.05 | 3 | 116 | 139 | 102 | 31 | 87.6 | 20.4 | 79 | 28 | 0.9 |
| 4 | 54 | MALE | 22.6 | 5 | 112 | 140 | 109 | 41 | 77.2 | 21.8 | 92 | 28 | 0.7 |
| 5 | 39 | MALE | 23.4 | 3 | 185 | 193 | 114 | 31 | 139.2 | 22.8 | 91 | 17 | 1.1 |
| 6 | 47 | MALE | 27 | 3 | 185 | 149 | 76 | 31 | 102.8 | 15.2 | 87 | 38 | 0.7 |
| 7 | 34 | MALE | 21.5 | 6 | 92 | 141 | 92 | 41 | 81.6 | 18.4 | 101 | 25 | 0.9 |
| 8 | 43 | FEMALE | 27.8 | 4 | 171 | 191 | 86 | 49 | 124.8 | 17.2 | 108 | 33 | 1.2 |
| 9 | 42 | MALE | 30.4 | 5 | 168 | 185 | 103 | 37 | 127.4 | 20.6 | 98 | 29 | 0.8 |
| 10 | 29 | FEMALE | 34.2 | 6 | 96 | 180 | 101 | 39 | 120.8 | 20.2 | 84 | 20 | 0.9 |
| 11 | 31 | FEMALE | 30.2 | 3 | 175 | 167 | 115 | 36 | 108 | 23 | 89 | 28 | 0.7 |
| 12 | 31 | FEMALE | 24.2 | 3 | 98 | 191 | 107 | 37 | 132.6 | 21.4 | 94 | 34 | 1.1 |
| 13 | 51 | FEMALE | 34.2 | 3 | 161 | 147 | 90 | 35 | 94 | 18 | 104 | 20 | 0.9 |
| 14 | 44 | MALE | 21.2 | 2 | 190 | 167 | 94 | 32 | 116.2 | 18.8 | 80 | 28 | 0.8 |
| 15 | 29 | FEMALE | 21.2 | 1 | 190 | 159 | 101 | 40 | 98.8 | 20.2 | 90 | 38 | 1.2 |
| 16 | 48 | FEMALE | 21.2 | 1 | 105 | 195 | 110 | 53 | 120 | 22 | 82 | 25 | 0.6 |
| 17 | 27 | MALE | 30.2 | 1 | 81 | 135 | 94 | 32 | 84.2 | 18.8 | 88 | 28 | 0.7 |
| 18 | 70 | MALE | 21.7 | 3 | 157 | 161 | 86 | 31 | 112.8 | 17.2 | 85 | 28 | 0.8 |
| 19 | 47 | FEMALE | 20 | 5 | 105 | 208 | 111 | 51 | 134.8 | 22.2 | 90 | 28 | 0.9 |
| 20 | 48 | MALE | 21.3 | 2 | 157 | 183 | 117 | 46 | 113.6 | 23.4 | 92 | 22 | 0.7 |
| 21 | 67 | MALE | 25.6 | 4 | 54 | 155 | 78 | 35 | 104.4 | 15.6 | 92 | 32 | 1.1 |
| 22 | 57 | FEMALE | 22.6 | 5 | 185 | 192 | 87 | 44 | 130.6 | 17.4 | 89 | 28 | 0.7 |
| 23 | 30 | FEMALE | 30.4 | 5 | 110 | 173 | 81 | 34 | 122.8 | 16.2 | 100 | 29 | 0.9 |
| 24 | 58 | MALE | 20.8 | 1 | 130 | 165 | 77 | 36 | 113.6 | 15.4 | 83 | 35 | 0.8 |
| 25 | 40 | FEMALE | 20.8 | 3 | 155 | 160 | 135 | 41 | 92 | 27 | 82 | 31 | 0.8 |
| 26 | 65 | MALE | 26.9 | 4 | 147 | 187 | 105 | 34 | 132 | 21 | 102 | 38 | 0.7 |
| 27 | 35 | FEMALE | 20.5 | 5 | 135 | 194 | 102 | 35 | 138.6 | 20.4 | 101 | 25 | 0.8 |
| 28 | 62 | MALE | 32 | 2 | 175 | 171 | 76 | 39 | 116.8 | 15.2 | 99 | 29 | 1.2 |
| 29 | 49 | MALE | 23.7 | 4 | 187 | 184 | 112 | 35 | 126.6 | 22.4 | 0.7 | 18 | 0.7 |
| 30 | 45 | MALE | 30.2 | 2 | 172 | 169 | 87 | 33 | 118.6 | 17.4 | 84 | 38 | 0.6 |

# KEY TO MASTER CHART

HsCRP - high sensitivity C reactive protein

LP(a) – Lipoprotein(a)

HDL-c – High density lipoprotein cholesterol

LDL-c - Low density lipoprotein cholesterol

VLDL-c – Very Low density lipoprotein cholesterol

BMI – Body Mass Index

P/Y – Pack years

A- Absent

P- Present

# PROFORMA

NAME: IP NO:

AGE: DOA:

SEX: SE CLASS:LOW/MIDDLE/HIGH

ADDRESS:

OCCUPATION:

PRESENTING COMPLAINTS DURATION

COUGH

SPUTUM PRODUCTION

DIFFICULTY IN BREATHING

WHEEZE

HEMOPTYSIS

CHEST PAIN

FEVER

LEG SWELLING

OTHERS (SPECIFY)

RISK FACTORS & TRIGGERS:

SMOKING

AIR POLLUTION LIKE SMOKE/DUST

RECURRENT URT

ATOPY/ALLERGY

SMOKING HISTORY:

ACTIVE: CIGAR/CIGARETTE/BEEDI

AGE AT WHICH SMOKING STARTED

INTENSITY OF SMOKING IN PACK YEARS

PASSIVE:

FATHER/HUSBAND/SON

HOUSE HOLD SMOKING

PACK YEARS OF SMOKING

FAMILY HISTORY:

BRONCHIAL ASTHMA/COPD

OTHER DISEASES:

PHT/SHT/DM/CAHD/VALVULAR HEART DISEASE/CONNECTIVE TISSUE

DISEASE

TREATMENT HISTORY

GENERAL EXAMINATION

ANAEMIA: PRESENT/ABSENT

POLYCYTHEMIA: PRESENT/ABSENT

CYANOSIS: PRESENT/ABSENT

PURSE LIP BREATHING: PRESENT/ABSENT

USE OF ACCESSORY MUSCLES OF BREATHING: PRESENT/ABSENT

LYMPH NODE ENLARGEMENT: PRESENT/ABSENT

PEDAL EDEMA: PRESENT/ABSENT

JUGULAR VENOUS PULSE:

VITALS:

PR: /MIN BP: mm/Hg RR: /MIN

SPO2: % TEMP: BMI:

SYSTEMIC EXAMINATION

CARDIO VASCULAR SYSTEM:

CENTRAL NERVOUS SYSTEM:

ABDOMINAL SYSYTEM:

CONGESTIVE HEPATOMEGALY: PRESENT/ABSENT

RESPIRATORY SYSTEM:

INSPECTION:

PALPATION:

PERCUSSION:

AUSCULTATION:

**INVESTIGATIONS**

| | | | |
|---|---|---|---|
| URINE R/E: | SUGAR | ALBUMIN | DEPOSITS |
| CBC: | Hb | ESR | |
| | TC | DC | PLATELETS |

FBS :

SR.UREA :

SR.CREATININE :

ECG :

CXR :

Hs – CRP :

LIPOPROTEIN(a) :

LIPID PROFILE :-

TOTAL CHOLESTEROL :

TGL :

HDL-C :

LDL-C :

VLDL-C :

SPIROMETRY:

| GOLD STAGING | FEV 1 | FEV1/FVC |
|---|---|---|
| STAGE 1 | | |
| STAGE 2 | | |
| STAGE 3 | | |
| STAGE 4 | | |

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